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HOT-WATER TREATMENT OF GLADIOLUS CORMELS FOR THE ERADICATION OF *FUSARIUM OXYSPORUM* F. *GLADIOLI*[1]

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INTRODUCTION

AN OBSTACLE to the use of pathogen-free gladiolus corms as a primary disease control is the universal contamination of commercial varieties, which makes it very difficult to obtain such clean stock by simple selection. As Buxton (1955*a*)[4] has pointed out, "Once the Fusarium has become established in a stock of corms by infection from the soil, eradication is almost impossible." However, two alternative methods remain for obtaining clean stock: 1) New pathogen- and virus-free varieties may be developed from seed. This is a slow and expensive procedure, even when the factor of actual resistance is not involved. 2) Methods may be devised for commercially freeing present varieties from the major pathogens. Some studies with this promising approach are presented in this paper. Of course, with either method, it is necessary to protect the stock from reinfection.

Of the available means for completely freeing planting stock of disease organisms, treatment with chemicals has not been effective because many of the pathogens are carried internally in deep lesions or in the vascular elements. Heat therapy has been investigated by several workers with little success. Massey (1916) found that tube cultures of the hard-rot fungus (*Septoria gladioli* Pass.) and dry-rot fungus (*Stromatinia gladioli* (Drayt.) Whet.) were killed at about 122° F for 10 minutes, and that medium-size corms were not materially harmed by dry heat of 122° F for 90 minutes, or by hot water of 122° F for 30 minutes. However, when corms with dry rot and hard rot were treated as above on the day they were dug, neither pathogen

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

was eliminated, and some heat injury was evident. It should be pointed out that the temperatures were too low, and that for the hot-water treatment "a half-bushel galvanized iron measure was used, the heat being supplied by an oil-stove flame." In our experience it is not possible to adequately treat plant material in so small a volume of water (slightly more than 3 gallons) heated in this way. Finally, corms have been found by several workers to be quite sensitive to heat.

Unpublished data[5] of Lucia McCulloch on hot-water treatments of corms in August to November, 1923, showed that temperatures of 95° to 140° F for 10 to 60 minutes failed to eliminate *Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans. in any combination which did not likewise kill the corms.

Drayton (1929) tested several hot-water and hot-fungicide treatments for elimination of the dry-rot and hard-rot pathogens from corms. Apparently hot water of 125° to 130° F for 30 minutes was ineffective. Treatment for 15 minutes in 5 or 10 per cent Semesan or Uspulun solutions heated to 122° F was more effective, but was considered impracticable because of cost and inconvenience.

Simmons (1949) found that use of high temperatures for curing corms tended to break dormancy. He found that 90 per cent of those exposed to dry heat of 135° F for 15 minutes produced shoots.

Gould (1954) tried curing corms at 110°, 120°, and 130° F for 10 hours, but produced heat injury and apparently did not reduce the incidence of *Botrytis gladiolorum* Timmermans.

Gladiolus growers in San Diego County have treated corms and cormels in hot water of 112° F for 2½ hours just before planting to control root-knot nematode. It was observed that the hot-water treatments induced a more uniform sprouting of the cormels, and treatment at 115° F for 30 minutes was, therefore, adopted for treating cormels to improve germination.

Several floricultural pathologists have also informed the writers of unsuccessful tests (unpublished) with heat treatment of corms to free them of pathogens.

There was, thus, only a suggestion that gladiolus corms and cormels would tolerate heat therapy, and no evidence that pathogens could be successfully eradicated without host injury by such treatment, when Roistacher (1951) began studies in 1950 on heat treatment of gladiolus cormels for the eradication of internal pathogens. This lack of success may have been due to the heat sensitivity of corms as compared with cormels, but the condition of the material treated must also have been involved. Indeed, it is not unlikely that some of the failure of treated stock to grow may actually have been a result of deepened dormancy, rather than death.

The control of Fusarium yellows and basal rot (*Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans.), of the important gladiolus diseases, probably would be most improved by the existence of an available pathogen-free stock. There are several reasons for this: 1) The losses from the disease are increasing despite careful application of present control procedures. The

[5] Courtesy of W. D. McClellan, Horticultural Crops Research Branch, U. S. Department of Agriculture, Beltsville, Maryland.

Fusarium yellows and basal-rot complex is recognized generally as the most serious disease of gladiolus. Magie (1953) reported an average annual loss in Florida of $200 an acre, and stated that nearly 200 million Picardy corms had been shipped into Florida since 1944, mainly to replace rotted corms. Magie (1954) later estimated that 40 to 50 million corms had rotted in Florida each year since 1948, about 97 per cent due to Fusarium. This was enough to plant approximately 1,000 acres or about an eighth of the total annual Florida crop. The situation in California is also serious. Growers have moved from infested to clean land, until available new areas are now almost gone. In the warmer sections of the state, Fusarium builds up very rapidly after introduction with infested planting stock. 2) Satisfactory resistant gladiolus varieties adapted to local growing areas are not available in many colors. This type of control, usually effective against Fusarium wilt diseases, is not much help against gladiolus yellows. 3) The causal Fusarium will persist in infested soil for many years, although the inoculum potential may decline somewhat after three to four years. 4) The Fusarium invades the vascular system of the plant, and because of this internal location is not effectively controlled by chemical treatment.

The cormel seemed to offer more favorable material for hot-water treatment than the corm. An investigation was undertaken to determine the heat tolerance of the gladiolus cormel, and of pathogenic fungi inside it. By increasing the margin between the exposure for thermal inactivation of cormel and of parasite, a method for the production of pathogen-free gladiolus stock was provided. Factors which would increase the thermal tolerance of the cormel were also studied.

THERMAL RELATIONS OF CORMELS

The following procedure was employed in the tests reported here. Cormels, in lots of 25 to 100, were placed in labeled cheese cloth or plastic screen sacks. All bags to be treated at the same temperature were placed in a large screen box which was submerged in the hot-water bath.

A tank designed for hot-water seed treatment (Type 7 of Baker and Roistacher, 1957) was used. The 200 to 250 gallons of water in the tank were heated by escaping steam, and the temperature held within $\pm \frac{1}{4}°$ F by manually regulating the steam inflow valve. The water was kept vigorously circulating by a pump to prevent stratification. Temperature readings were taken on two thermometers in each test.

Immediately after a specified time of submergence the bags of cormels were cooled by immersion in cool water to precisely terminate the treatment. They were then planted in pots in the greenhouse, and emergence counts taken every week.

Thermal Death Point of Cormels

Cormels of the variety Picardy were treated at 106°, 113°, 122°, 131°, and 140° F for 10, 20, and 30 minutes. Three replicates of 100 cormels each were planted in a U. C.-type soil mix in 18 × 18-inch redwood flats on benches in the glasshouse. Figure 1 shows graphically the percentage emergence of sample groups of cormels treated at 113°, 122°, and 131° F for 30 minutes, and at 140° F for 10 minutes.

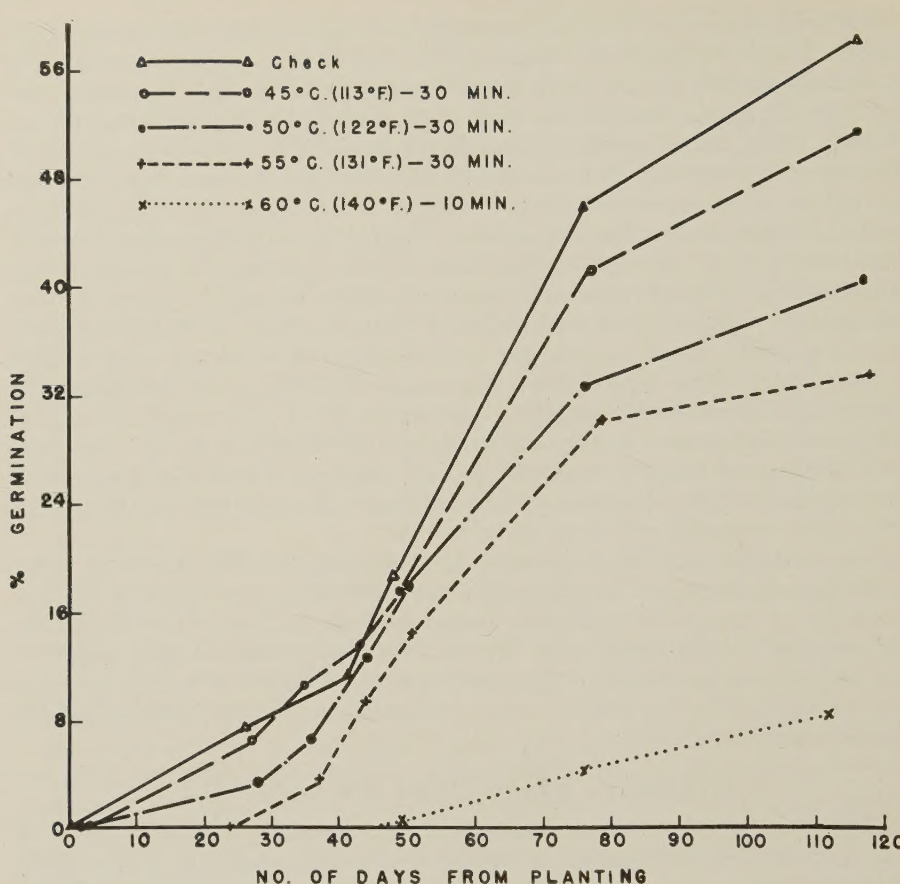

Fig. 1. The effect of four different hot-water treatments on the time and percentage of germination of Picardy cormels. Each curve represents an average of three replicates totaling 300 cormels.

An inactivation temperature between 131° and 140° F for 20 to 30 minutes was indicated by these results. The 30-minute immersion was adopted as standard for all subsequent tests.

The dormancy problem was also revealed in these tests. At the end of 110 days, cormels were still sprouting in all treated lots. It was deemed advisable to investigate some methods of breaking dormancy to see whether the germination time could be shortened.

Relationship of Hot-water Treatment to Cormel Dormancy

Test 1. An experiment was planned to study the possibility of breaking cormel dormancy in order to shorten the period of emergence after hot-water treatments. Cormels of three varieties were obtained locally in October, 1950:

1) Spotlight, dug on September 1, 1950, and soaked in water for a few days to wash away the soil. The cormels were badly diseased and a majority had cracked outer husks.

Fig. 2. Effect of heat treatment on cormels of variety Spotlight 67 days after planting. Untreated check at left; treated in hot water (128° F) for 30 minutes, in center; treated in hot 5 per cent ethyl alchohol for 30 minutes, at right. Treated lots stored at 40° F for 3 weeks prior to planting.

2) Myrna Fay, dug October 20, 1950. The cormels appeared sound and healthy, but approximately 40 per cent had cracked outer husks.
3) Miss Wisconsin, dug October 20, 1950. The cormels had a very hard, thick, and intact outer husk.

A safe hot-water bath (128° F for 30 minutes) was used, followed by various treatments to break dormancy, as follows:

Hot-water treatments

1. Check
2. Hot water at 128° F, 30 minutes
3. 5 per cent ethyl alcohol at 128° F, 30 minutes

Subsequent dormancy treatments

A. Check
B. Cold storage at 40° F for 3 weeks
C. Ethylene chlorohydrin (1.6 per cent) at 70° F, 48 hours
D. Ethylene chlorohydrin as above, followed by a 2 minute dip in 95 per cent ethyl alcohol[6]

[6] Strydom (1949) found a short dip in 95 per cent alcohol effective in breaking dormancy of corms.

All combinations of hot-water and subsequent treatment were tested, except 3C, which left 11 combinations. Since the number of cormels was limited, 25 of each variety were used per treatment, without replication.

After 96 days the soil was carefully washed from cormels of the varieties Myrna Fay and Miss Wisconsin, and the numbers dead were recorded. Ungerminated cormels were stripped of their husks, replanted, and observed after 60 days. The results are recorded in table 2.

TABLE 1

EFFECT OF VARIOUS TREATMENTS ON CORMELS OF SPOTLIGHT VARIETY 61, 88, AND 120 DAYS AFTER PLANTING IN SOIL, AS SHOWN BY EMERGENCE, DORMANCY, DEATH, AND ROOT CONDITION. FIGURES INDICATE THE NUMBER OF CORMELS OUT OF 25 IN EACH TREATMENT*

Treatment	Plant condition	Posttreatment manipulation			
		Check	Cold storage	Ethylene chlorohydrin	Ethylene chlorohydrin plus alcohol
No heat treatment (check)	Emergence, 61 days	2	11	16	4
	88 days	2	18	21	16
	120 days†	16	18	16	15
	Dormant in soil, 120 days	5	1	0	2
	Dead in soil, 120 days	9	6	17	21
	Condition of roots‡	R	W–Y	R	R
128° F for 30 min. in water	Emergence, 61 days	5	12	14	4
	88 days	22	16	17	7
	120 days†	23	20	19	12
	Dormant in soil, 120 days	0	1	1	0
	Dead in soil, 120 days	2	6	5	14
	Condition of roots‡	W	W	W–Y	W–Y
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	16	17	..	1
	88 days	19	21	..	10
	120 days†	22	21	..	14
	Dormant in soil, 120 days	1	1	..	1
	Dead in soil, 120 days	4	3	..	10
	Condition of roots‡	W	W	..	W

* See also figure 3.
† Includes those sprouted in the soil.
‡ R = Rotted; W–Y = white roots with some yellowing; W = white roots without yellowing.

After 120 days, cormels of the variety Spotlight were carefully removed and examined for germination, rotting, and root condition. The results are presented in table 1 and figure 3. Because most of the cormels of this variety had already sprouted, they were not peeled and replanted.

The results of this experiment show a number of interesting points. Since there were no replications, higher order interactions were used as an estimate of error. Significant effects of treatment were as follows:

1) Heat-treated cormels of the variety Spotlight, in addition to germinating more rapidly, produced clean white roots. By contrast, the untreated cormels had decayed roots, and the series treated with hot water and then with ethylene chlorohydrin were somewhat yellowed. The cause of the yellowing

was not determined. It was indicated that the hot-water treatment produced clean stock in series not subsequently treated with ethylene chlorohydrin

2) In the variety Spotlight, hot-water treatment broke cormel dormancy (fig. 2). After 88 days, 22 out of 25 cormels had emerged, as against two in the controls. The results with Myrna Fay and Miss Wisconsin were less pronounced.

That hot-water treatment will break dormancy of vegetative structures has been repeatedly demonstrated. Treatment of sugar-cane stem cuttings in hot water (122° to 125.6° F) for 20 minutes causes the rapid development

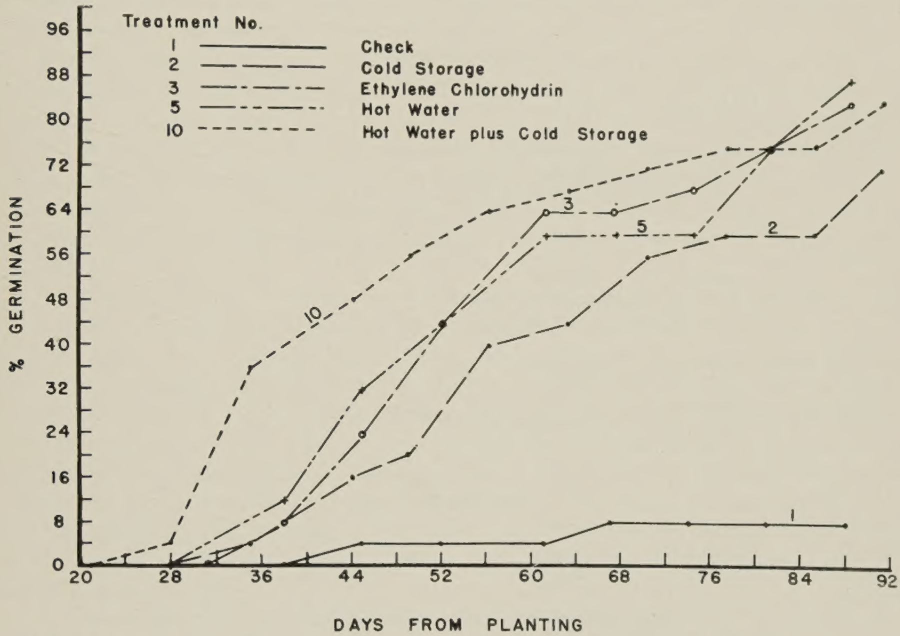

Fig. 3. Rate of sprouting of Spotlight cormels treated in four ways to break dormancy: storage at 40° F for 3 weeks; treatment with ethylene chlorohydrin; treatment with hot water at 128° F for 30 minutes; treatment with hot water at 128° F for 30 minutes, followed by 3 weeks storage at 40° F. Each curve represents 25 cormels. (See also table 1.)

of lateral buds, probably because of a lowered auxin level (Brandes and Klaphaak, 1923; Brandes and van Overbeek, 1948). Similar results have been obtained with a wide range of plants. Denny (1930) reported that sprouting of gladiolus corms was enhanced to some extent by warm storage (86° F for 3 weeks) during the later stages of dormancy. Loomis (1934) found that storage at 104° F for 1 week, at 95° F for 2 weeks, at 86° F for 4 weeks, or at 77° F for 6 weeks accelerated corm germination of all gladiolus varieties tested. As mentioned above, hot-water treatment of cormels at 115° F for 30 minutes is an accepted practice of growers in San Diego County to break dormancy and cause more uniform sprouting.

3) Although all three varieties were dug at approximately the same time, they differed in degree of dormancy. This is shown by their different reac-

TABLE 2

EFFECT OF VARIOUS TREATMENTS ON CORMELS OF VARIETIES MYRNA FAY AND MISS WISCONSIN 61, 96, AND 156 DAYS AFTER PLANTING IN SOIL, AS SHOWN BY EMERGENCE AND DEATH. FIGURES INDICATE THE NUMBER OF CORMELS OUT OF 25 IN EACH TREATMENT

Treatment	Plant condition	Posttreatment manipulation			
		Check	Cold storage	Ethylene chlorohydrin	Ethylene chlorohydrin plus alcohol
	Variety Myrna Fay				
No heat treatment (check)	Emergence, 61 days	0	8	3	3
	96 days	4	12	8	8
	Dead in soil, 96 days	2	1	1	2
	Additional emergence 60 days after peeling (156 days after planting)	19	12	14	7
128° F for 30 min. in water	Emergence, 61 days	4	10	4	1
	96 days	9	11	7	2
	Dead in soil, 96 days	11	5	5	7
	Additional emergence 60 days after peeling (156 days after planting)	5	9	7	3
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	8	11	..	1
	96 days	10	14	..	2
	Dead in soil, 96 days	7	7	..	13
	Additional emergence 60 days after peeling (156 days after planting)	8	1	..	3
	Variety Miss Wisconsin				
No heat treatment (check)	Emergence, 61 days	1	4	1	1
	96 days	2	4	2	1
	Dead in soil, 96 days	0	1	1	5
	Additional emergence 60 days after peeling (156 days after planting)	20	12	21	19
128° F for 30 min. in water	Emergence, 61 days	0	0	1	0
	96 days	1	0	1	0
	Dead in soil, 96 days	8	6	2	6
	Additional emergence 60 days after peeling (156 days after planting)	16	13	8	9
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	0	0	..	0
	96 days	1	0	..	0
	Dead in soil, 96 days	2	10	..	9
	Additional emergence 60 days after peeling (156 days after planting)	13	10	..	9

tions to the various dormancy-breaking treatments. The variety Miss Wisconsin would not sprout sufficiently, regardless of the treatment used. However, a sister lot of cormels of this variety planted after the removal of their outer husks gave rapid sprouting (fig. 9).

4) Cold storage of untreated cormels also enhanced the process of sprouting (tables 1 and 2). This is in accord with the results of commercial practice,

and the work of Denny and Miller (1935). Cold storage after hot-water treatment was most effective in enhancing sprouting of Myrna Fay and Spotlight cormels (fig. 3, hot water followed by cold storage).

5) Ethylene chlorohydrin was also effective in breaking dormancy, but it tended to increase discoloration and rotting of the corms and roots. Ethylene chlorohydrin plus a dip in 95 per cent alcohol appeared to be too severe a treatment.

6) Treatment in a 5 per cent ethyl alcohol bath at 128° F increased sprouting in the checks and, when followed by cold storage, produced vigorous and healthy plants (fig. 2). This was evident in top growth, the size and vigor of the new corms, and whiteness of roots.

Test 2. This experiment was designed to answer four questions: 1) What temperature between 131° and 140° F applied for 30 minutes will inactivate cormels? 2) Does this critical temperature vary for different varieties? 3) What is the comparative response of the host to a hot 5 per cent alcohol bath and a hot-water treatment? This reaction is distinct from the effect on dormancy of alcohol at a lower temperature (128° F; see above). 4) What is the effect of a period of cold storage prior to hot-water treatment of cormels?

Cormels for this experiment were harvested in late August, 1950, at Oceanside, California[7], and held in open storage sheds until late November. There were six varieties: Myrna Fay, Miss Wisconsin, True Love, Easter Parade, Beneson, and Variety X (unlabeled). The cormels were soaked in a 1:1,000 mercuric chloride solution for 2 hours, and rinsed in running water for 20 minutes. After drying, cormels of each of the six varieties were divided into two equal lots. One was placed in 40° F storage, and the other prepared for immediate hot-water treatment. The latter group was placed, in lots of 25 cormels each, in 72 small, labeled, plastic screen bags. The 24 treatments were arranged as follows for each of the six varieties:

- No cold storage:
  - Presoak in 5 per cent alcohol at 94° F for 20 hours
    - Untreated check
    - Treated in 5 per cent alcohol for 30 minutes at 131°, 133°, 135°, 137°, and 140° F
  - No presoak
    - Untreated check
    - Treated in hot water for 30 minutes at 131°, 133°, 135°, 137°, and 140° F
- Cold storage (40° F) for 3 weeks:
  - Presoak in 5 per cent alcohol at 94° F for 20 hours
    - Untreated check
    - Treated in 5 per cent alcohol for 30 minutes at 131°, 133°, 135°, 137°, and 140° F
  - No presoak
    - Untreated check
    - Treated in hot water for 30 minutes at 131°, 133°, 135°, 137°, and 140° F

[7] The authors are indebted to Mr. Edwin Frazee, Oceanside, for supplying cormels for many of these tests.

The cormels of the alcohol series were placed in tightly sealed glass jars of 5 per cent ethyl alcohol, and held at 94° F for 20 hours. This was to augment the effect of the subsequent treatments in hot 5 per cent alcohol.

The series treated in hot water were held dry at room temperature for 20 hours before treatment.

After 3 weeks the cormels that had been held in cold storage were removed and treated in the same ways as the above series.

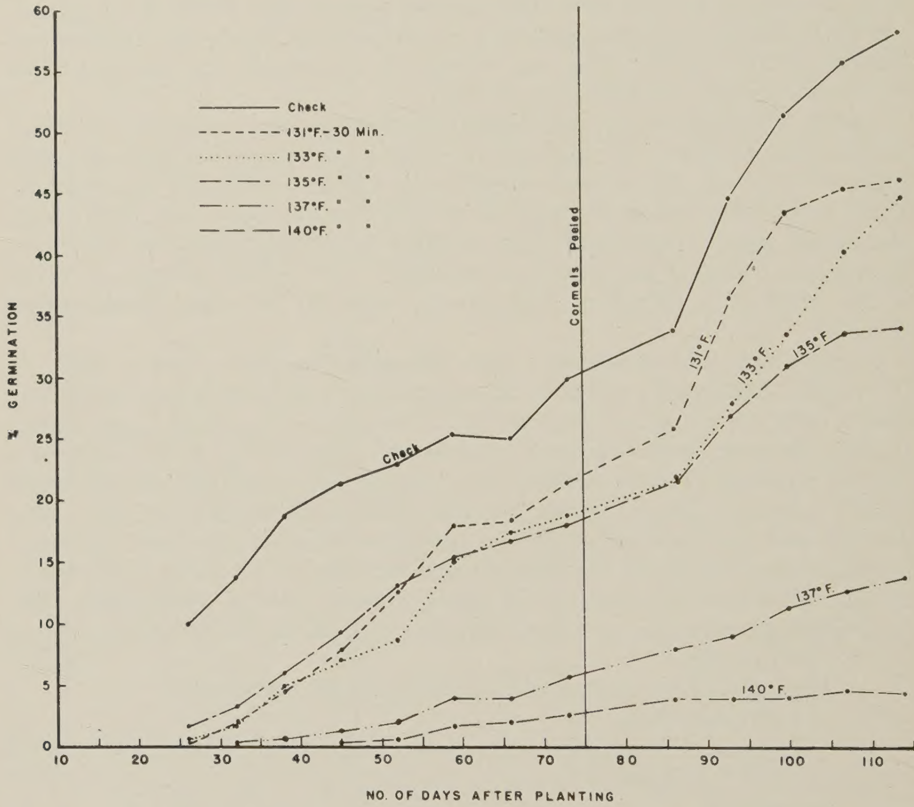

Fig. 4. The effect of heat treatment at five different temperatures on semidormant cormels. Each curve represents 300 cormels of six varieties, and includes both alcohol and water series. Cormels were dug 73 days after treatment, peeled, and replanted for 47 days.

The temperature of the water or alcohol baths had a fluctuation of $\pm \frac{1}{4}$° F during treatments. The bags of cormels were placed in cold water for 3 minutes immediately after treatment. Each lot of 25 cormels was then planted in a 4-inch pot and placed in the glasshouse. Emergence counts were recorded weekly. Since the number of variety-treatments and limited number of cormels precluded replications, higher order interactions supplied an estimate of error.

The original data were arranged in several ways to evaluate the effect of the various factors on germination. The effect of heat treatment at five different temperatures on semidormant (noncold storage) cormels is pre-

sented in figure 4; the effect on nondormant (cold storage) cormels is shown in figure 5. The effect of cold storage on susceptibility of cormels to heat treatment is obtained by comparing figures 4 and 5, and by comparing figures 6 and 7. The comparison of treating semidormant cormels in hot water and in hot 5 per cent alcohol is shown in figure 6; the effect on nondormant cormels is shown in figure 7. The effect of hot-water treatments at five temperatures in inducing cormel dormancy is shown in figure 8. The data shown

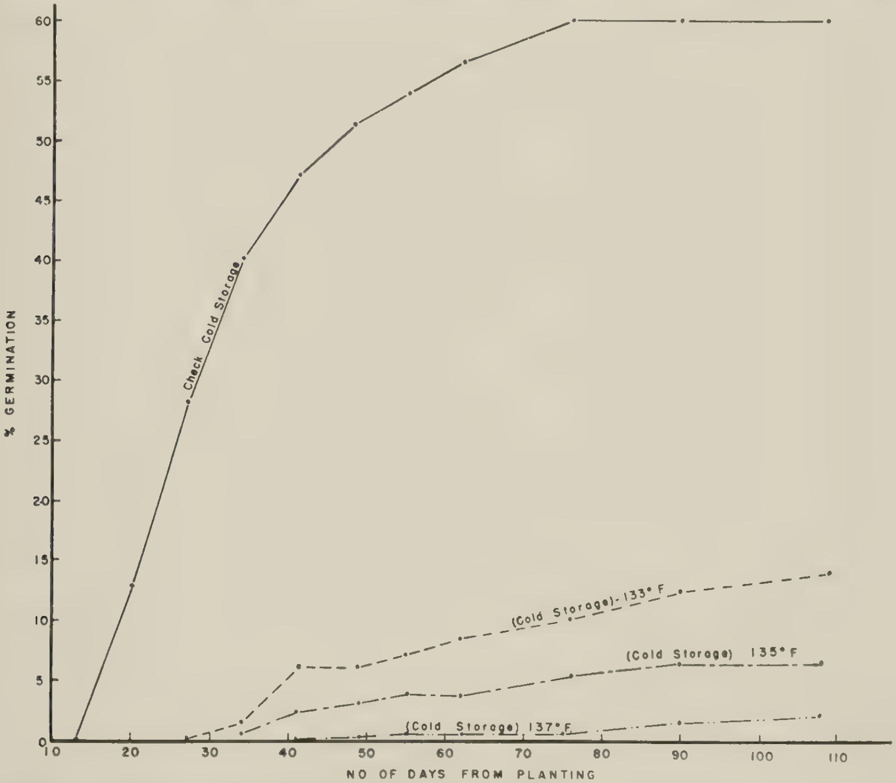

Fig. 5. The effect of heat treatment at three different temperatures on cormels whose dormancy had been broken by storage at 40° F for 3 weeks. Each curve represents 300 cormels of six varieties, and includes both alcohol and water series.

graphically in figures 4 and 5 for the combined totals of all six varieties and of the alcohol and water heat treatments, indicate that 135° F for 30 minutes was the highest temperature that could be tolerated without undue injury or delayed germination of semidormant cormels. These results are in expansion of an earlier abstract (Roistacher, 1951).

The germination of cormels held in cold storage before hot-water treatment at temperatures of 131° to 140° F was seriously depressed.

Treating in a 5 per cent alcohol bath after a 5 per cent alcohol soak impaired sprouting somewhat, but not seriously enough to prevent its use if it facilitated killing the pathogens. Actually, soaking and treating cormels in

a 5 per cent alcohol bath did not materially reduce sprouting below the levels attained by material treated in hot water. This is shown in figures 6 and 7, based on the combined totals of all six varieties and all temperatures.

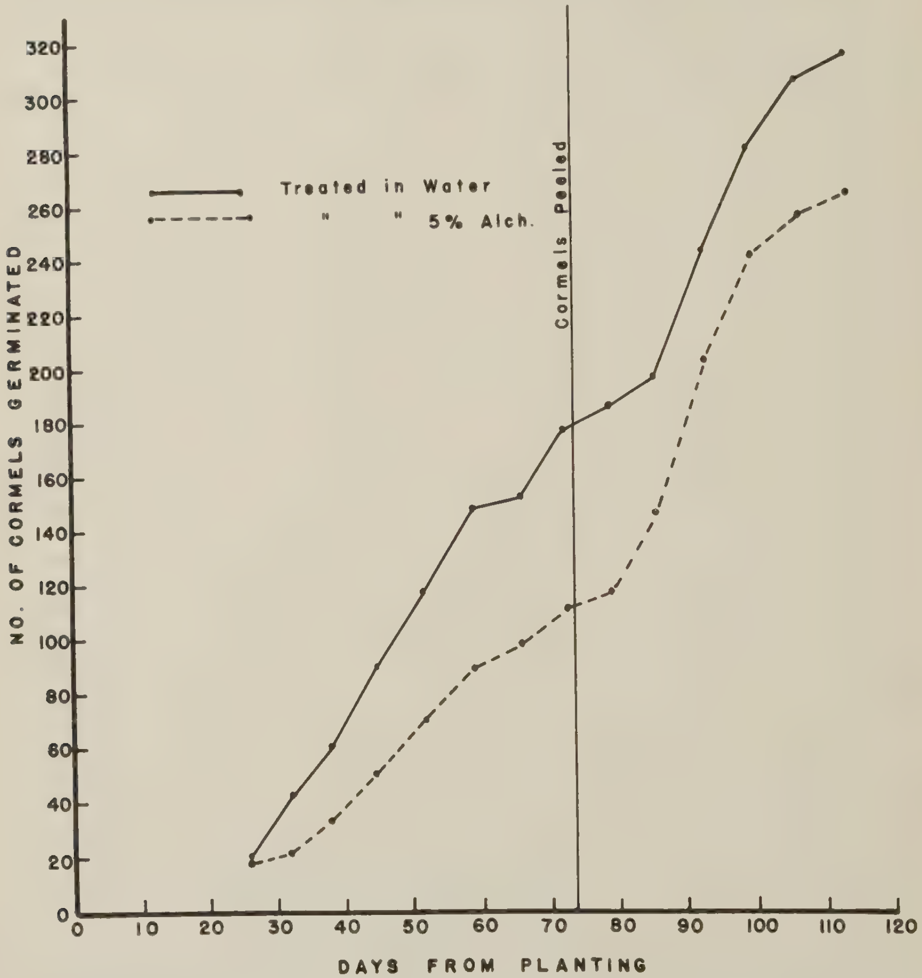

Fig. 6. The comparative effect on the germination of semidormant cormels of treatment in hot water (30 minutes at 131° to 140° F) and in hot alcohol (20-hour presoak in 5 per cent ethyl alcohol at 94° F, followed by 30 minutes at 131° to 140° F). Each curve represents 900 cormels of six varieties, and includes the untreated check and those treated at 131°, 133°, 135°, 137°, and 140° F. Cormels were dug 73 days after treatment, peeled, and replanted for 47 days.

Different varieties showed about the same general critical temperatures between 135° and 140° F. However, some of them were thrown into a state of prolonged dormancy by the high temperature, rather than killed by it. Thus, 50 per cent of the cormels treated at 140° F were found to be alive and turgid 142 days after planting. Seventy-three days after planting,

cormels in the noncold storage treated lots were carefully screened from the soil, and the number found dead was recorded (fig. 8). The unsprouted but turgid cormels were replanted after the outer husks were removed. A marked increase in sprouting occurred following removal of the husks from cormels of Myrna Fay, Easter Parade, and Variety X. It did not materially enhance sprouting of those cormels treated at 140° F. The variety Miss Wisconsin, which was relatively unaffected by all dormancy-breaking treatments in earlier experiments (test 1), sprouted readily when the outer husk was removed (fig. 9).

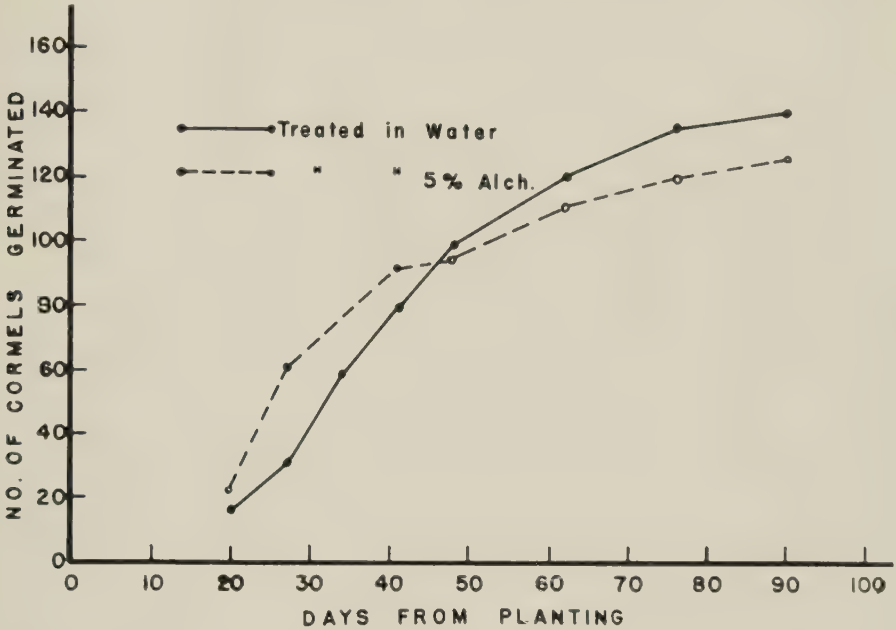

Fig. 7. The comparative effect of treatment in hot water (30 minutes at 131° to 140° F) and in hot alcohol (20-hour presoak in 5 per cent ethyl alcohol at 94° F, followed by 30 minutes at 131° to 140° F) on germination of cormels whose dormancy had been broken by storage at 40° F for 3 weeks. Each curve represents 900 cormels of six varieties, and includes the untreated check and those treated at 131°, 133°, 135°, 137°, and 140° F.

The Effect on Cormel Dormancy of Cracking or Removing the Husk

Test 3. Dormancy in cormels, as in seeds, may be affected by the thickness of the outer husk. If these are cracked or removed, dormancy may be broken (fig. 9).

An experiment was designed to test the effect on germination of cracking the outer husk of the cormels before heat treatment. The cormels used were dug at Oceanside, California, in December, and stored there in an open shed until March, when they were treated. The varieties used were Elizabeth the Queen, Leading Lady, Margaret Beaton, and Valeria. There were four

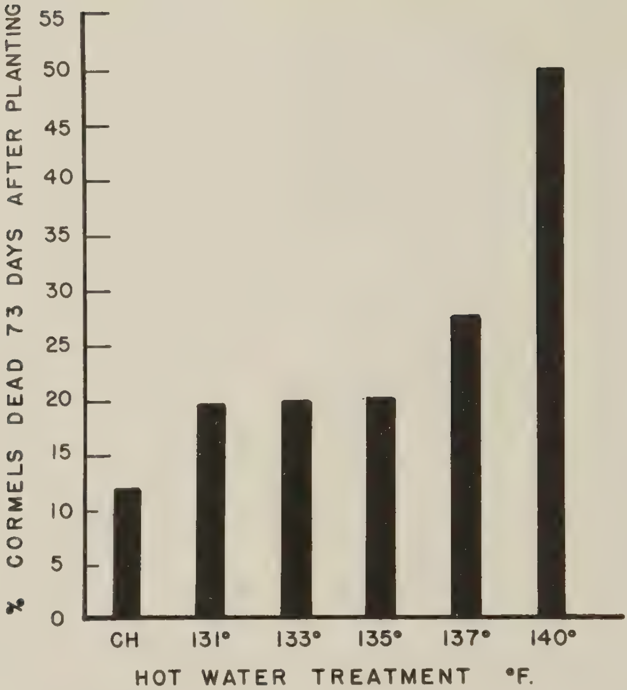

Fig. 8. The percentages of cormels found dead in the series untreated (check) and treated (at five different temperatures for 30 minutes) in hot water or alcohol, 73 days after planting. The cormels were from the semidormant group that had not been held in cold storage. Each bar represents 300 cormels of six varieties. Note the increasing number of cormels found dead in the series above 135°, but that only 50 per cent were killed, even at 140° F.

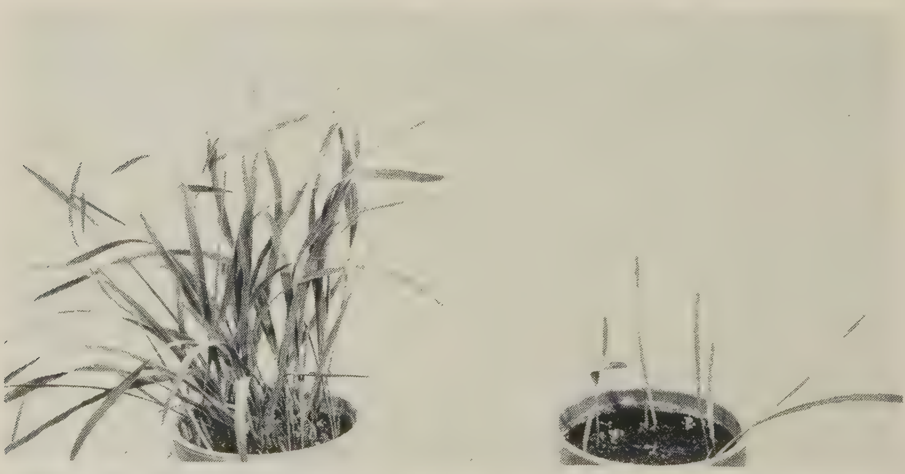

Fig. 9. The effect on germination of removing the outer husk of cormels of the variety Miss Wisconsin (left), compared with unpeeled cormels (right) 60 days from planting.

TABLE 3

MEAN PERCENTAGES OF GERMINATION OF CORMELS IN FOUR REPLICATES (200 CORMELS) OF FOUR VARIETIES 69 DAYS AFTER PLANTING IN SOIL, SHOWING THE EFFECT OF CRACKING OF THE HUSK ON GERMINATION AND SENSITIVITY TO HEAT TREATMENT

Variety	Husks not cracked (check)	Husks cracked before hot-water treatment at				
		Check	120° F	125° F	130° F	135° F
Elizabeth the Queen	22	62	54	54	44	23
Leading Lady	1	74	68	67	29	15
Margaret Beaton	8	68	73	60	26	7
Valeria	5	58	48	54	27	9

TABLE 4

MEAN PERCENTAGE OF GERMINATED, DORMANT, AND DEAD CORMELS IN FOUR REPLICATES (100 CORMELS) OF TWO VARIETIES 60 DAYS AFTER PLANTING IN SOIL, SHOWING THE EFFECTS OF HOT-WATER TREATMENT AND SUBSEQUENT CRACKING OF THE HUSKS

Treatment	Husks not cracked			Husks cracked following hot-water treatment		
	Sprouted	Dormant	Dead	Sprouted	Dormant	Dead
Variety Valeria						
Check	23	76	1	84	1	15
128° F for 30 min	55	35	10	47	5	48
131° F for 30 min	65	26	9	55	0	44
135° F for 30 min	9	83	9	22	9	69
138° F for 30 min	34	65	1	32	3	65
Variety Leading Lady						
Check	47	48	5	72	4	24
128° F for 30 min	24	70	6	54	19	27
131° F for 30 min	35	62	3	39	11	50
135° F for 30 min	61	36	3	56	2	42
138° F for 30 min	2	87	11	8	8	84

replicates of 50 cormels each in the following treatments: husk not cracked, untreated (check); husk cracked, untreated; husk cracked, treated at 120°, 125°, 130°, or 135° F for 30 minutes. Cormels were rinsed in cold water after treatment and planted in 6-inch pots. Germination counts were taken at weekly intervals. The percentage germination 69 days after planting is shown in table 3.

Cracking the husks enhanced sprouting of all four varieties, both without heat treatment and when followed by treatment at temperatures up to 130° F. Treatment at 135° F did not materially increase sprouting over the uncracked checks, and significantly reduced it compared to the cracked checks.

The results raised three questions: 1) What would be the effect of cracking the husks after hot-water treatment? 2) How would these cormels compare with uncracked heat-treated cormels of the same lots? 3) How many of the nonsprouted cormels were dead or still dormant in the soil?

Test 4. This experiment used cormels of the same lots of two varieties, Leading Lady and Valeria, used in test 3. The material had been held at room temperature. There were four replicates of 25 cormels each in the following treatments: untreated check; hot-water treatment at 128°, 131°, 135°, and 138° F for 30 minutes. Since the tetrazolium test (Roistacher, Bald, and Baker, 1953, 1957) indicated that these cormels were dormant, a series at 138° F was included to see whether they would survive treatment. An overnight soak in cool water preceded hot-water treatment. Following the heat treatment the cormels were cooled in water, dried, and the outer husks of half of each lot were cracked. The various lots were then planted in pots in a glasshouse, and emergence counts recorded weekly. The results 60 days later are recorded in table 4.

The results show that cracking the husks *after* hot-water treatment increased the lethal effect of all temperatures from 128° to 138° F but did not materially affect the percentage sprouted after 60 days. It was undetermined whether cormels dormant at 60 days were still capable of germinating. In the untreated checks, cracking of the husks materially increased sprouting, as was the case in test 3.

Dormant cormels of both varieties withstood a temperature as high as 138° F. Only 1 per cent were found to be dead in the variety Valeria, and 11 per cent in Leading Lady, in cormels with uncracked husks treated at 138° F. This indicates the value of the tetrazolium test in judging whether cormels are in a state most tolerant of hot-water treatment.

THERMAL DEATH POINTS OF FUNGI IN CORMELS

In conjunction with the above experiments on the thermal tolerance of cormels, experiments were conducted on the thermal tolerance of two important pathogens when growing in cormels.

Gassner (1933) found that the addition of 2 to 5 per cent ethyl alcohol to the water bath reduced the temperature necessary to kill the loose smut fungus (*Ustilago nuda* (Jens.) Rostr.) in wheat seed by 9° to 18° F. This effect apparently was from action on the pathogen rather than the host. Alcohol was accordingly included in some of the treatments against gladiolus pathogens. Its effect on the heat tolerance and dormancy of the cormel was presented in the preceding section of this paper.

Fusarium oxysporum f. *gladioli.*

The fungus *Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans. was selected for the most detailed study, for reasons outlined in the "Introduction." The primary objective was to determine the inactivation temperature of the fungus when growing in the cormel, and to compare it with that of the cormel.

Twelve separate trials were conducted with the following general procedure: The Fusarium inoculum used in trials 1 through 8 was obtained

from lesions of naturally infected corms from southern California. Single-spore isolates were made at intervals to maintain the wild type of the fungus, and these were used as inoculum. For trial 9, the isolates were obtained from four different sources. Mass-transfer cultures of these were maintained and used as inoculum. Cultures for trials 10 and 11 were obtained from an artificially inoculated cormel in trial 9, which had survived hot-water treatment at 135° F for 30 minutes. In trial 12, all available isolates were mixed for use in a spore suspension.

Procedure for wounding and inoculation in trials 1 to 9 consisted of pricking the cormels with a coarse flattened needle, and pressing fragments of an agar culture into the wounds. In trials 10 to 12, the procedure was modified by cracking the outer husks in addition to puncturing, and submerging the wounded cormels in a spore suspension of the inoculum for 10 minutes at room temperature. In either method, the inoculated cormels were placed in Petri dishes and incubated for 4 to 6 days at 80° F, which rotted the cormels approximately half way through. In trials 2, 3, and 10, the cormels were incubated for an additional 10 to 21 days at 94° F to stimulate formation of the resistant chlamydospores. Preliminary testing had indicated this was the best treatment to induce chlamydospore production.

After wounding, inoculation, and incubation the cormels were placed in small, labeled, plastic screen bags and treated in hot water or hot 5 per cent alcohol. After treatment, cormels were cooled in running tap water for a few seconds prior to plating out.

The procedure for determining the presence of living Fusarium after hot-water treatment was as follows: Each cormel was aseptically bisected through the wounded area, placed in a Clorox solution (diluted 1:12) for 1 minute, and transferred to a Petri plate containing potato-dextrose agar. Four to 13 cormels were placed in each plate, varying with the quantity used per trial. The plates were incubated for 1 week at 80° F, and then examined for Fusarium. Plates which were sterile at that time still showed no development of Fusarium when held for as long as 1 month. Results for each of the 12 trials are presented in table 5, and summarized in table 6.

In table 6 the following results are shown:

1) Presoaking is necessary for successful eradication of the fungus. The importance of presoaking before hot-water treatment to eliminate internal air pockets had been previously shown (Baker and Davis, 1950) in treating nasturtium seed for eradication of *Heterosporium tropaeoli* Bond. Unless cormels are presoaked, the Fusarium apparently can survive even at the higher temperatures. It is not unlikely that there are air pockets between the husk and the cormels, as there are between the pericarp and seed of nasturtium.

2) The addition of 5 per cent alcohol to the treatment bath may improve the effectiveness against Fusarium. It may make possible the use of lower temperatures, as suggested by Gassner (1933). Results obtained here suggest a trend favorable to this concept. Hot-water treatments at 128° to 136° F of nonpresoaked cormels gave 169 out of 342 with Fusarium, while hot 5 per cent alcohol treatments at the same temperatures showed 0 out of 30. However, in presoaked cormels the corresponding figures were 27 out of 354 and 1 out of 570.

TABLE 5

EFFECTIVENESS OF HOT WATER, PLUS VARIOUS PRETREATMENTS, IN ERADICATING *FUSARIUM OXYSPORUM* F. *GLADIOLI* FROM CORMELS OF EIGHT VARIETIES OF GLADIOLUS. UNLESS OTHERWISE NOTED, THE CORMELS NOT PRESOAKED AND THOSE PRESOAKED IN WATER WERE ALSO TREATED IN WATER, AND THOSE PRESOAKED IN ALCOHOL WERE TREATED IN ALCOHOL

Variety	Cormels per treatment	Days incubated at		Presoak treatment	Heat treatment		Per cent cormels with living Fusarium
		80° F	94° F		Temperature (° F)	Time (min.)	
				Trial 1			
Picardy	30	6	0	none	check	..	100
	10	6	0	none	113	30	100
					122	20	100
					122	30	100
					131	10	100
					131	20	100
					131	30	30
					136	20	100
					136	30	0
				Trial 2			
Picardy	4	4	10	water, 94° F, overnight	check	..	100
					128	60	100
					131	30	75
					131	45	50
					133	30	50
					133	45	50
					135	30	0
					135	45	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	60	25
					131	30	0
					131	45	75
					133	30	0
					133	45	0
					135	30	0
					135	45	0
				Trial 3			
Picardy	4	4	24	none	check	..	100
					131	30	100
					133	30	25
					135	30	100
				water, 94° F, overnight	check	..	75
					131	30	100
					133	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight*	check	..	100
					131	30	0
					133	30	50
					135	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at 80° F	Days incubated at 94° F	Presoak treatment	Heat treatment: Temperature (° F)	Heat treatment: Time (min.)	Per cent cormels with living Fusarium
Trial 4							
Picardy	4	4	35	water, 94° F, overnight	check	..	75
					131	30	75
					133	30	50
				5 per cent alcohol, 94° F, overnight	check	..	75
					131	30	0
					133	30	0
Trial 5							
Miss Wisconsin	22	4	0	none	134	30	32
Variety X	22	4	0	none	134	30	36
Easter Parade	21	4	0	none	134	30	48
Beneson	22	4	0	none	134	30	64
Myrna Fay	25	4	0	none	134	30	68
True Love	17	4	0	none	134	30	71
Miss Wisconsin	22	4	0	none	135	30	59
Variety X	22	4	0	none	135	30	37
Easter Parade	23	4	0	none	135	30	17
Beneson	22	4	0	none	135	30	68
Myrna Fay	25	4	0	none	135	30	72
True Love	17	4	0	none	135	30	65
Trial 6							
Miss Wisconsin	20	4	0	5 per cent alcohol, 94° F, overnight	135	30	0
Variety X	20	4	0		135	30	0
Easter Parade	20	4	0		135	30	0
Beneson	20	4	0		135	30	0
Myrna Fay	20	4	0		135	30	0
True Love	20	4	0		135	30	0
Trial 7							
Miss Wisconsin	20	4	0	5 per cent alcohol, 94° F, overnight	133	30	0
Variety X	20	4	0		133	30	0
Easter Parade	20	4	0		133	30	0
Beneson	20	4	0		133	30	0
Myrna Fay	20	4	0		133	30	0
True Love	20	4	0		133	30	0
Trial 8							
All six varieties	60	4	0	water, 94° F, overnight	check	..	98
Miss Wisconsin	10	4	0		133	30	0
Variety X	10	4	0		133	30	10
Easter Parade	10	4	0		133	30	0
Beneson	10	4	0		133	30	0
Myrna Fay	10	4	0		133	30	0
True Love	10	4	0		133	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at 80° F	Days incubated at 94° F	Presoak treatment	Heat treatment: Temperature (° F)	Heat treatment: Time (min.)	Per cent cormels with living Fusarium
				Trial 8—*Continued*			
All six varieties	60	4	0	5 per cent alcohol, 94° F, overnight	check	..	90
Miss Wisconsin	10	4	0		133	30	0
Variety X	10	4	0		133	30	0
Easter Parade	10	4	0		133	30	0
Beneson	10	4	0		133	30	0
Myrna Fay	10	4	0		133	30	0
True Love	10	4	0		133	30	0
				Trial 9			
Valeria	20†	4	0	water, 94° F, overnight	check	..	100
	40†	4	0		131	30	2.5‡
	40†	4	0		133	30	0
	40†	4	0		135	30	0
	20†	4	0	5 per cent alcohol, 94° F, overnight	check	..	100
	40†	4	0		131	30	0
	40†	4	0		133	30	0
	40†	4	0		135	30	2.5‡
				Trial 10§			
Valeria	10	4	24	water, 94° F, overnight	check	..	100
					131	30	70
					133	30	10
					135	30	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					131	30	0
					133	30	0
					135	30	0
				Trial 11§			
Valeria	5	4	0	none	check	..	100
	15	4	0		131	30	0
	15	4	0		135	30	0
	15	4	0		131‖	30	0
	15	4	0		135‖	30	0
	20	4	0	water, 94° F, overnight	check	..	100
	20	4	0		131	30	0
	20	4	0		135	30	0
	20	4	0	5 per cent alcohol, 94° F, overnight	check	..	100
	20	4	0		131	30	0
	20	4	0		135	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at 80° F	Days incubated at 94° F	Presoak treatment	Heat treatment: Temperature (° F)	Heat treatment: Time (min.)	Per cent cormels with living Fusarium
				Trial 12			
Valeria¶	10	5	0	water, 94° F, overnight	check	..	90
					128	30	10
					131	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0
Valeria**	10	5	0	water, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0

* This alcohol presoak series was treated in hot water.
† Total for four lots of cormels infected with Fusarium from four different sources.
‡ These escapes came from a single inoculum source.
§ Cormels wounded and inoculated by dipping in a spore suspension from trial 9.
|| Treated in 5 per cent alcohol, rather than water.
¶ Cormels had been held 14 months in storage.
** Cormels freshly dug.

3) The thermal death point of the Fusarium, following a presoak in water or 5 per cent alcohol, lies between 128° and 135° F for a 30-minute interval. Dormant cormels treated at the upper levels of this range may be expected to survive, and to be relatively free from the fungus.

The question might fairly be raised whether various strains of the gladiolus Fusarium could have different levels of thermal tolerance. For example, the known tendency of some isolates to produce large numbers of chlamydospores might provide greater heat tolerance. Some of the isolates used in these studies produced chlamydospores, and trials 2, 3, and 10 were manipulated to give maximum numbers of these structures. In addition approximately 15 to 20 different isolates were used in these tests. Trials 10 and 11 involved an isolate which had survived 135° F in trial 9; the fungus did not survive 135° F in trial 10 or 133° F in trial 11.

Also some uncertainty has existed whether there is more than one distinct type of Fusarium disease of gladiolus, and whether these might be caused by different species or forms. Forsberg (1955) has recently studied this question and concluded that the 40 isolates from three types of diseases could not be distinguished morphologically, by response to temperature or to fungicides, by various growth characteristics on artificial media, or by type of disease produced when inoculated into the host. Single isolates were found

to produce more than one type of disease. He considered that all the forms should be included in *F. oxysporum* f. *gladioli*. Buxton (1955*a*) also found that single isolates of *F. oxysporum* f. *gladioli* would cause both yellows and corm rot. The type of symptom produced was determined both by the gladiolus variety and by the given isolate of the fungus. Buxton (1955*b*) suggested that the variability in the latter might result from the recombination of existing heterocaryons. Bruhn (1955) found that his isolates caused both corm rot and vascular yellows diseases and, for this and other reasons, placed them all in *F. oxysporum* f. *gladioli*.

TABLE 6

SUMMARY OF THE 12 TRIALS FROM TABLE 5, SHOWING RATIO OF CORMELS WITH LIVING FUSARIUM TO THE TOTAL NUMBER, FOLLOWING VARIOUS HOT-WATER TREATMENTS

Treatment temperature and time	Cormels not presoaked			Cormels presoaked in water or alcohol		
	Treated in 5 per cent alcohol	Treated in water	Totals	Treated in 5 per cent alcohol	Treated in water	Totals
Untreated check			39/39			271/284
113° F—30 min.	....	10/10	10/10	...	....	....
122° F—30 min.	....	10/10	10/10	.....	....	....
128° F—30 min.	....	....	....	0/20	1/20	1/40
131° F—30 min.	0/15	7/29	7/44	0/98	18/106	18/204
133° F—30 min.	....	1/4	1/4	0/238	8/126	8/364
134° F—30 min.	....	68/129	68/129	....	....	....
135° F—30 min.	0/15	73/150	73/165	1/214	0/102	1/316
136° F—30 min.	....	0/10	0/10	....	....	....
Totals	0/30	169/342	169/372	1/570	27/354	28/924

The well-known variability of Fusaria might make possible the future selection of an isolate with higher thermal tolerance if heat treatment became general, much as DDT-resistant house flies have appeared. However, this circumstance has not developed with bacteria or other microörganisms that have long been subjected to minimal lethal temperatures (*e.g.*, pasteurization of milk). Everything considered, it is unlikely that Fusaria of such thermal tolerance as to render the treatment ineffectual will appear. However, since cormels in a proper state of dormancy can often tolerate temperatures well above 135° F (*e.g.*, figs. 1, 4, and 8, and table 4), there is still some margin of safety.

Stromatinia gladioli

In March, 1951, dead gladiolus stems were collected that had large numbers of sclerotia of the dry-rot fungus, *Stromatinia gladioli*. These were held in cold storage for 2 weeks and then treated with hot water to determine the thermal death point of the sclerotia.

Basal leaf strips ¼ inch wide by ¾ inch long, heavily dotted with sclerotia, were cut from many different plants and randomly placed in five plastic screen bags. These were then treated as follows: untreated check; treated at

125°, 130°, 135°, or 140° F for 30 minutes in hot water. After treatment each sample was placed in Clorox solution (diluted 1:12) for approximately 1 minute. Five leaf strips were placed in each of three Petri dishes of potato-dextrose agar, and held at room temperature for 1 week.

The sclerotia of the fungus were killed by the hot-water bath at 125° F for 30 minutes, the lowest temperature tried, as well as at 130°, 135°, and 140° F. The untreated sclerotia germinated readily. It was of interest that nematodes noticed in the three check plates were not found in any of the plates with tissue treated at 125° F or above.

DISCUSSION

The determination of a safe temperature for hot-water treatment of gladiolus cormels involves understanding and manipulating a number of factors. Since the thermal death point of the cormel lies close to that of the most important pathogen, *Fusarium oxysporum* f. *gladioli*, treatment temperatures close to the maximum for the host must be used if the organism is to be eradicated.

Successful heat therapy presupposes a safe margin between the thermal death point of host and pathogen. Little difficulty is encountered if this difference is large. Where the margin is small it can often be increased in several ways:

1) Selecting the most favorable type of material for treatment. The use of cormels rather than the less tolerant corms, as in this study, improves chances of survival.

2) Selecting material in the state of dormancy most tolerant of treatment, or manipulation of the material to produce that state. Cormels made nondormant by cold storage (figs. 4 to 7) or by cracking their outer husks cannot stand high temperatures (table 3), and may be killed by temperatures of 131° F or above. However, if cormels are held under dry, warm conditions, and are found by a tetrazolium test to be dormant, they can withstand hot water temperatures as high as 138° F for 30 minutes without undue reduction of germination (table 4). This is in line with the results of Ryan (1955), who found that corms grown at temperatures above 59° F had a deeper rest than those grown intermittently at or below 50° F for the final few weeks before harvest. The treatment of semidormant summer-grown cormels from warm soil, rather than nondormant winter-grown cormels, similarly improves the chances of survival and germination (Bald and Markley, 1955). The lack of success from hot-water treatment of cormels grown in northern states with comparatively cool soil may be due to their lack of dormancy. The killing action of hot water on cormels is apparently associated with the metabolic rate at time of treatment. Thus, nondormant cormels are in a relatively high state of metabolic activity, and are readily killed at the lower temperatures.

3) Selecting the proper environmental conditions may:

a) Increase the thermal tolerance of the host plants (see 2 above).

b) Increase the susceptibility of the pathogen to heat treatment. The pre-soak treatment of the cormels may accomplish this in part by initiating fungus development, although the elimination of heat-insulating air pockets must also surely be involved. The addition of alcohol to the hot-water bath apparently decreases heat tolerance of some fungi. Gassner

(1933) found that the effect of heat on the wheat loose smut fungus in seed depended on the exclusion of oxygen, and thought that the intermediate products of host metabolism under these conditions killed the pathogen. He thought that the addition of alcohol to the hot bath augmented this action. This idea was indirectly supported by Tyner (1953) and Russell and Tyner (1954), who found that a soak in water on temperature-time schedules ranging from only 66° F for 80 hours to 86° F for 35 hours, eradicated the same fungus. Wallen and Skolko (1953) found that the incidence of *Ascochyta pisi* Lib. in pea seed was also reduced by an 18-hour soak in cool water, apparently from antibiotic substances produced by the bacterial flora, which was increased by the soak. It is not yet certain what mechanism or mechanisms may be involved in these effects.

Under our experimental conditions, by manipulation of such factors as dormancy, presoaking, and the addition of substances to the treating bath, the margin between inactivation temperatures of the pathogen and host may be increased. The pathogen may then be eradicated without significant injury to the host. The method has now moved into successful commercial application (Bald and Markley, 1955; Bald, 1956; Bald, Ferguson, and Markley, 1956; Ferguson and Markley, 1955; Magie, 1956).

SUMMARY

1. The thermal inactivation temperature of dormant gladiolus cormels treated in hot water for 30 minutes lies between 135° and 140° F. Six varieties reacted about the same toward this critical temperature range.

2. The thermal inactivation temperature by hot water of *Fusarium oxysporum* f. *gladioli* inside the cormel lies between 128° and 135° F. *Stromatinia gladioli* sclerotia are killed by 125° F for 30 minutes in basal leaf tissue.

3. Although the margin between the thermal death point of host and pathogen is small, manipulation of certain factors may so increase it that successful commercial treatment is made completely practical.

a) Since cormels appear to be much more tolerant of heat than are corms, they have been exclusively used for heat treatments.

b) The level of dormancy of the cormel strongly affected the thermal inactivation point. Nondormant cormels did not survive treatment at 131° F, whereas dormant cormels survived temperatures as high as 140° F. The use of the tetrazolium method for estimating the dormancy of cormels is, therefore, a valuable tool in reducing injury from heat treatment.

c) Presoaking the cormels before the hot-water bath increased effectiveness of treatment. Fusarium inside the cormels often survived treatment unless the overnight presoak was used.

d) The use of 5 per cent ethyl alcohol in the treatment bath increased the effectiveness in eradicating Fusarium.

4. A markedly increased sprouting was induced by removing the outer husk from the cormel 73 days after hot-water treatment, but cracking them before or immediately after treatment sharply reduced the number surviving at higher treatment temperatures.

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2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE AS AN INDICATOR OF GERMINABILITY AND DORMANCY OF GLADIOLUS CORMELS[1]

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INTRODUCTION

DURING TRIALS of hot-water treatment of gladiolus cormels (Roistacher, Baker, and Bald, 1957)[4] it appeared that a method for quickly determining the stage of dormancy of cormels might make it possible to treat them effectively without injury or without prolonging dormancy. The chemical 2,3,5-triphenyltetrazolium chloride was investigated as a possible indicator of cormel dormancy.

2,3,5-triphenyltetrazolium chloride[5] is a water-soluble colorless salt that forms insoluble carmine red triphenyl formazan when in a reduced state. It has been extensively investigated as an indicator of high metabolic activity in a variety of plant and animal tissues, since the property of turning red in active metabolizing tissue was first reported by Kuhn and Jerchel (1941).

It has had wide use on many kinds of seeds as an index of germinability, since the method was introduced for this purpose by Lakon (1942*a*,*b*). The literature on this use has been reviewed by Porter, Durrell, and Romm (1947), Flemion and Poole (1948), and Smith (1951). A good correlation generally has been noted between the appearance of red coloration in the embryos and the percentage germination in a given lot of seed, but numerous factors must be taken into account for successful commercial application to a specific plant. The general method has also been adapted to measure the damage (reduced germinability) to seeds from freezing (Goodsell, 1948; Bennett and Loomis, 1949; Parker, 1953), excessive heating (Fuchs and Beiler, 1943, 1948; Brewer, 1949; Macleod, 1950; Lambou, 1953), drying (Brewer, 1949; Parker, 1953), and cathode rays (Lambou, 1953), as well as probable ability to grow in cold soil (Germ and Kietreiber, 1954).

Tetrazolium salts have also been used to measure vitality of plant tissues other than seeds. Mattson, Jensen, and Dutcher (1947) found that TTC would stain tissue from the fleshy part of apples, oranges, and grapes, mushroom gills, carrot roots, white and sweet potatoes, young leaves, and stigmas and ovaries of pollinated flowers, as well as animal tissue. Waugh (1948) tested dormant twigs of several kinds of trees, and found that TTC stained the cambial layers. Twigs killed by heat gave no reaction. Dufrenoy and Pratt (1948) showed that the upper nodes of sugar cane stems reduced TTC.

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

[5] Referred to in this paper as TTC or tetrazolium.

The degree of blanching of edible parts of several vegetables from heat was measured with TTC by Morse (1949). Roberts (1950) investigated stem and root tissues of 35 species of monocots, dicots, and ferns for TTC-reducing activity. In dicots, apical meristems of terminal and lateral buds and roots, as well as some lateral meristems were stained; in monocots, intercalary meristems and nodes were stained. Parker (1953, 1955) demonstrated TTC-reducing activity for meristematic tissue of pine leaves, stems, and roots, and for root tips of corn and onion.

The staining of microörganisms by TTC has been demonstrated for algae (Dyar, 1953; Parker, 1953), bacteria (Kuhn and Jerchel, 1941; Huddleson and Baltzer, 1950; Somerson and Morton, 1953), yeasts (Kuhn and Jerchel, 1941; Gunz, 1949; Currier and Day, 1954), actinomycetes (Fults, Schaal, and Michaelson, 1949), and Penicillium (Fred and Knight, 1949). Plant tissues invaded by several viruses were also shown to reduce TTC (Beal, Preston, and Mitchell, 1955).

Because of the versatility of TTC for measuring metabolic vitality in normal and injured tissue, tests were conducted to see whether it could be used to measure: 1) the probable tolerance of gladiolus cormels to heat treatment, and 2) the level of dormancy of cormels. A technique was developed to evaluate the degree of reddening of cormel tissues under standard conditions (Roistacher, Bald, and Baker, 1953). It was subsequently used by Tsukamoto (1954) to indicate dormancy of corms as affected by heat treatment at 95° to 100° F. The present paper will report: 1) the background of the experimental procedure; 2) some correlations beween TTC reddening and germination; 3) association between cold storage, tetrazolium reddening, and cormel germination; 4) points to be considered in using TTC as a test for dormancy in gladiolus cormels.

A TECHNIQUE FOR MEASURING THE COLOR REACTION OF CORMEL TISSUE

At first, cormels were cut in half and dipped in a 1 per cent solution of TTC in tap water of pH 7.9.[6] A distinct reddening was noted in the meristematic region through the central portion of the cormel. Check cormels placed in water, or those boiled in water and placed in the TTC solution, did not show any reddening. A number of different varieties were tested and it was soon apparent that some varieties reddened faster than others. The technique was modified by placing the split cormels on a disk of moistened filter paper in a Petri dish in diffuse light. Numerical ratings were given to the various shades of pink and red formed in the central meristematic region and the parenchymatous tissues.

The following method was based on modifications of earlier tests and used as standard procedure for experiments reported here. The description is quoted from a previous summary of this work (Roistacher, Bald, and Baker, 1953).

"(1) A 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride is prepared and kept in a stoppered bottle in the dark at room temperature.

[6] The water should not have a pH of less than about 6.5, nor more than about 9.0 (Jámbor, 1954; Jámbor and Mester, 1955).

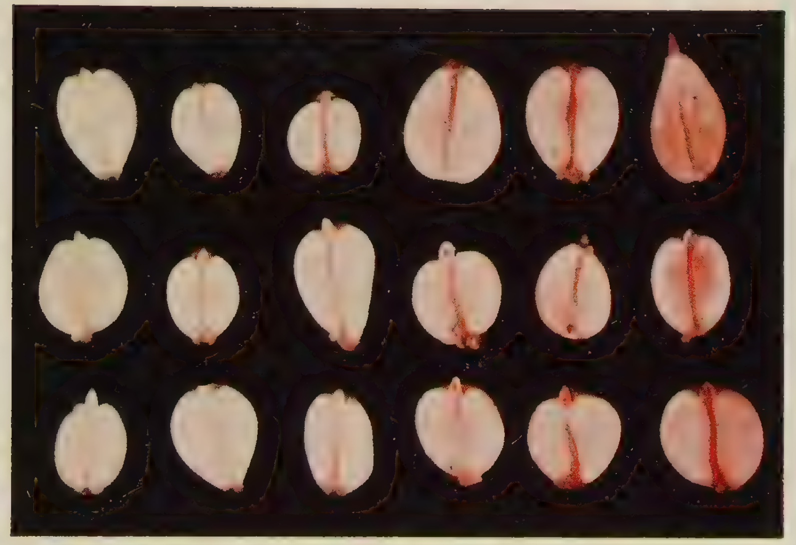

Fig. 1. Kodachrome photograph of cut surfaces of cormels exposed for 4 hours to TTC, showing the five numerical ratings based on the varying degrees of tetrazolium reddening. 0 at left, to 5 at right. Some varieties under certain conditions tend to give slightly more intense color than shown here.

(2) A single sheet of filter paper is placed in each of 2 Petri dishes and soaked with solution. The minimum amount required is about 2½ ml. per dish. The prepared dishes are kept in the dark if not used immediately. (3) Duplicate lots of 20 cormels, representative of the stock under test, are peeled (*i.e.*, the dry husk is removed) and cut longitudinally with a razor blade to expose the central vascular region on one or both halves. Recent tests suggest that peeling may be unnecessary if a little extra tetrazolium solution is put in the Petri dish to allow for absorption by the husks. (4) Each pair of half cormels is placed radially on the outside border of the

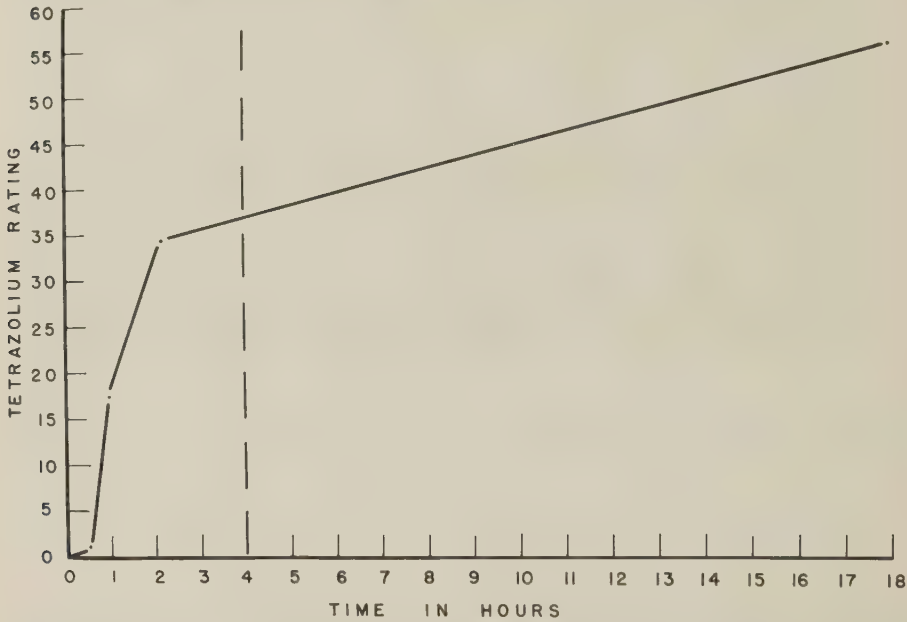

Fig. 2. The average intensity of reddening for eight gladiolus varieties over a period of 18 hours at 70° F. A 4-hour contact period between the cormel and the 1 per cent solution of the tetrazolium salt was adopted as standard in all subsequent tests.

filter paper, cut surface down. Twenty pairs about fill the perimeter of the paper. Two dishes are thus prepared, each from one of the duplicate lots of 20 cormels. (5) The dishes are placed in the dark at approximately 70° F to incubate for 4 hr. (6) The cormels are rated according to the extent and intensity of the red color developing in the vascular region through the center of the cormel, and any additional coloration throughout the storage tissues (fig. 1). The rating given for the whole cormel is that of the half cormel with the more pronounced coloration. The rating standard may be described as follows. A value of 0 is given to a cormel when there is no observable pink or red color from the dye; a faint coloration along the central vascular strand is rated 1; medium to strong color of the vascular strand, 2; strong color of the vascular strand plus some coloration throughout part of the parenchymatous storage tissues, 3; intense coloration of the central strand and medium coloration throughout most of the parenchymatous

tissues, 4; and intense coloration over the whole surface, 5. The sums of the ratings, which range between 0 and 100 for a single dish, give an estimate of the mean germinability of the lot of corms from which the duplicate samples are taken."

A 4-hour period was chosen as standard for incubating the cormel as a result of many tests on a number of varieties. Figure 2 shows the increase of color ratings as average values for eight varieties. After 3 hours further increase in intensity of coloring generally was very slow. A cormel showing a strong pink coloration at 4 hours appeared the same or perhaps only a little darker after 18 hours. It was concluded that if reddening was to occur, the majority of cormels would show it within 4 hours. Lambou (1953) reports using a 2 per cent solution and a 4-hour interval to achieve maximum reddening of cotton seed.

CONSISTENCY AND ACCURACY OF TETRAZOLIUM RATINGS

To test the uniformity of judgment by different observers, ratings for 40 cormels have been made by several people on a number of occasions, using the color chart (fig. 1) without further instructions. A very high degree of uniformity between ratings has been observed in all these tests.

In making records, the ratings were recorded in a table as follows, as each cormel was examined.

Sample 1					*Sample 2*			
3	3	1	2		4	1	4	2
1	2	2	2		3	3	2	2
2	3	2	3		2	2	3	1
4	3	3	2		5	3	2	3
2	2	5	3		3	4	5	2
12	13	13	12 = 50		17	13	16	10 = 56

By taking the mean of the two totals, a value was obtained (53), which gave an estimate of reddening for the sample on a 100-unit scale. This mean represents the total numerical rating for 20 cormels, and is used as a standard of comparison. Where more or less than 20 were used, the total was prorated to 20. The data from such a table were then assembled in a frequency series.

Degree of reddening	0	1	2	3	4	5
Number of cormels	0	4	16	13	4	3
	20			20		

Adding the frequencies for ratings 0 to 2 and 3 to 5 gave two values, separated on the basis of whether any coloration observed was restricted to the central vascular tissue, or whether it was present in both the vascular and parenchymatous tissues. The frequency series and the division of ratings into two categories gave a second method of estimating tetrazolium reddening, which has been particularly useful in judging cases where the frequency series was obviously skewed or heterogeneous.

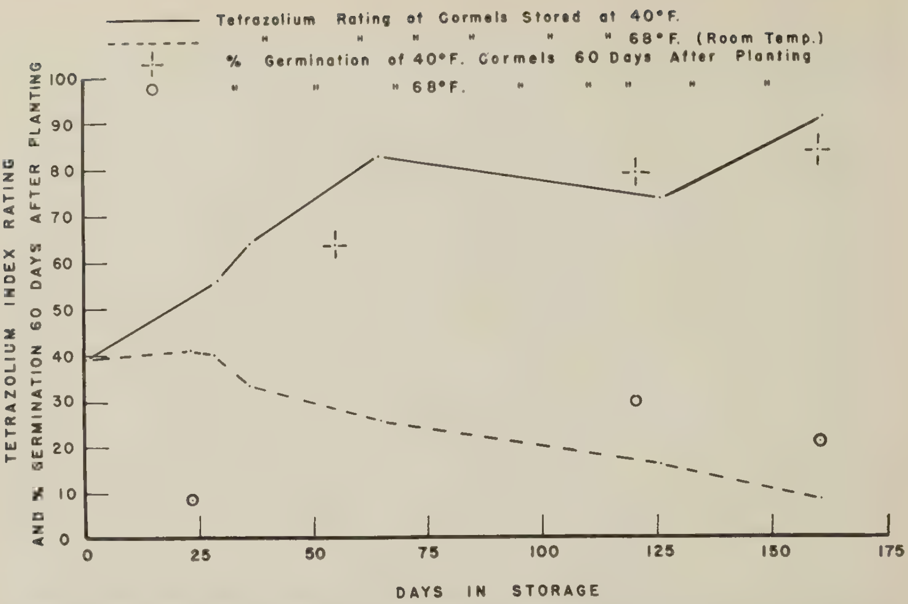

Fig. 3. The relationship between the degree of tetrazolium reddening and germination of dormant and nondormant cormels of variety Margaret Beaton. Cormels were divided into two lots—one held in cold storage (40° F) and the other at room temperature (68° F). Germination 60 days after planting is compared with the tetrazolium rating on the same scale.

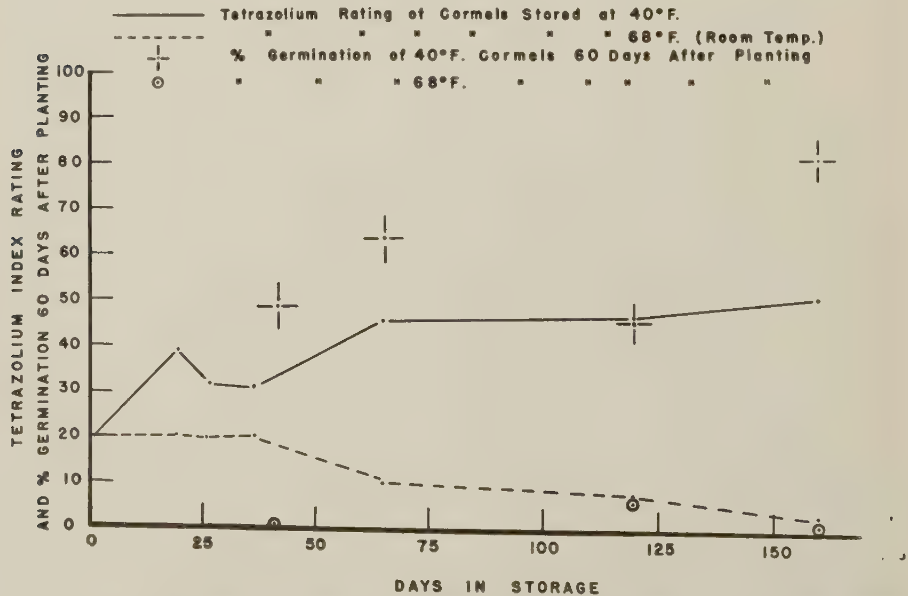

Fig. 4. The relationship between the degree of tetrazolium reddening and germination of dormant and nondormant cormels of variety Leading Lady. Cormels were divided into two lots—one held in cold storage (40° F) and the other at room temperature (68° F). Germination 60 days after planting is compared to the tetrazolium rating on the same scale.

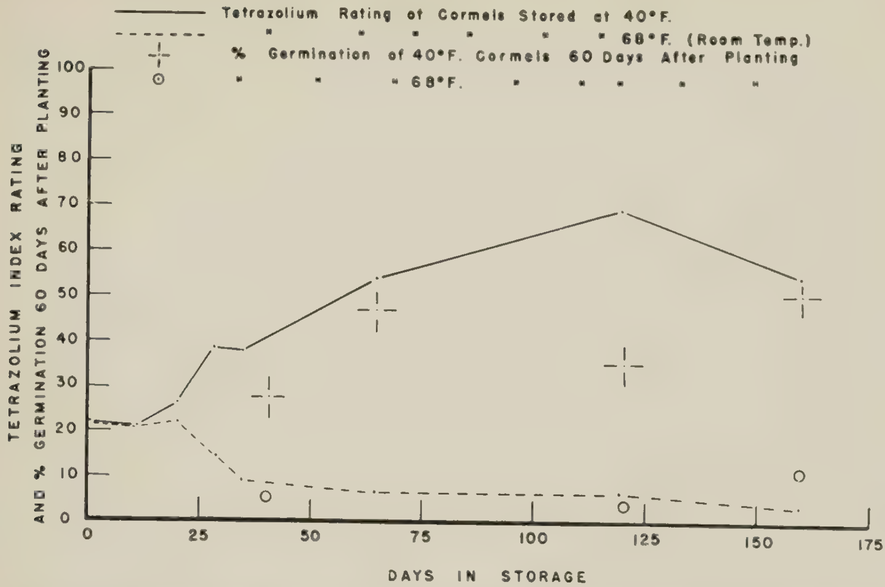

Fig. 5. The relationship between the degree of tetrazolium reddening and germination of dormant and nondormant cormels of variety Valeria. Cormels were divided into two lots—one held in cold storage (40° F) and the other at room temperature (68° F). Germination 60 days after planting is compared to the tetrazolium rating on the same scale. (See also tables 2 and 3.)

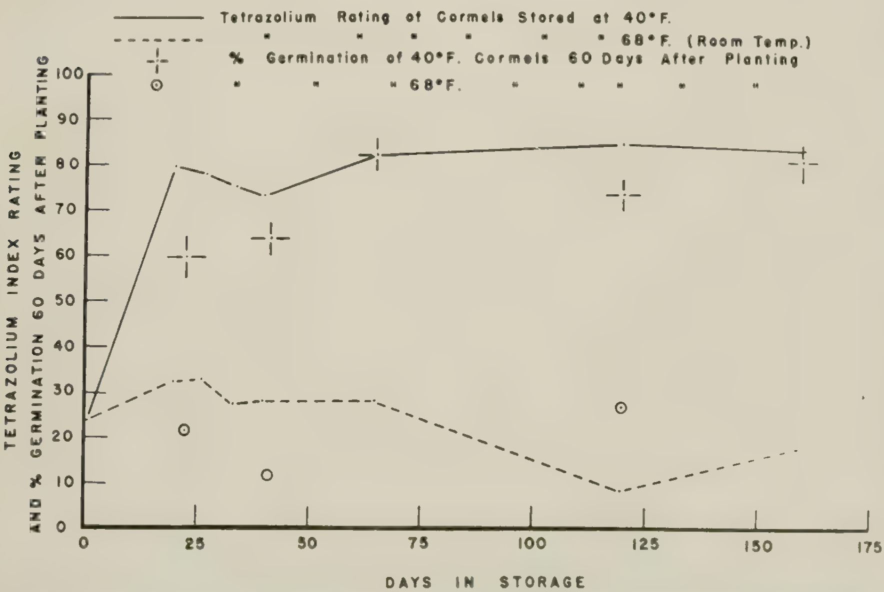

Fig. 6. The relationship between the degree of tetrazolium reddening and germination of dormant and nondormant cormels of variety Elizabeth the Queen. Cormels were divided into two lots—one held in cold storage (40° F) and the other at room temperature (68° F). Germination 60 days after planting is compared to the tetrazolium rating on the same scale.

Analysis of variance of the date from the table on page 689 gave an estimate of the accuracy of the mean rating. The least significant difference between ratings for two lots of 40 cormels having this degree of variability would be less than 10 units on the 100-unit scale.

Blocks	1	0.9	0.90
Error	38	42.2	1.11

Least significant difference between two lots of 40 cormels would be about 0.475 unit on the 5-unit scale.

The following analysis of variance indicates the variability to be expected in a series of seven experiments using four varieties. The data consisted of readings from 1,120 cormels of four varieties submitted to cold storage, and sampled on seven occasions. They represent parts of the data from a large experiment discussed later (figs. 3 to 6). There were significant differences between varieties and between occasions, and differences between varieties varied from one occasion to another. The residual variance, with 1,092 degrees of freedom, provided an estimate of the least significant difference between two lots of 40 cormels = 6.32 units on the 100-unit scale.

Varieties	3	138.332	46.111
Occasions	6	170.337	28.389
Varieties × Occasions	18	43.334	2.407
Error	1,092	546.665	0.501

Least significant difference between mean of two lots of 20 cormels, 6.316 units on the 100-unit scale.

These two instances provide examples of the accuracy of tetrazolium ratings for reasonably uniform material. The level of variability may be somewhat higher if less uniform samples of cormels are rated, but if samples are properly chosen it is seldom much higher than 10 per cent.

COMPARISON OF THE DEGREE OF TETRAZOLIUM REDDENING WITH GERMINATION POTENTIAL OF CORMELS

Preliminary Tests

Preliminary tests run in conjunction with hot-water treatments are shown in table 1. Where the majority of cormels has a low TTC rating (0 to 2), germination is low (variety Beneson); where most TTC ratings are high, germination is high (varieties Myrna Fay, 2; and one-year-old Picardy, 1) and where ratings are intermediate, germination is intermediate (varieties Myrna Fay, 1; and three-month-old Picardy, 2). A time interval of 60 days after planting was chosen as a standard to compare the per cent germination with the tetrazolium rating in further tests reported in this paper.

Correlations of TTC Rating with Germination

A series of experiments was begun to test the possibility of predicting cormel germination by the use of the tetrazolium test. Earlier tests revealed that if cormels showing a low rating were placed in cold storage, they would show a higher rating and a higher per cent germination after a time (table 1, variety Myrna Fay). An attempt was made to correlate the degree of redden-

TABLE 1

EXAMPLES OF SOME EARLY COMPARISONS BETWEEN TTC RATINGS AND GERMINABILITY

Variety	Tetrazolium test: Cormel treatment	Tetrazolium test: Frequency rating of single cormels: 0–2	Tetrazolium test: Frequency rating of single cormels: 3–5	Tetrazolium test: Rating for 20 cormels	Germination: Number cormels planted	Germination: Number days after planting	Germination: Germination per cent
Picardy 1	10 cormels, examined in early tests	2	8	80	100	45	61
Beneson	Dry, unpeeled	38	2	21	50*	45	4
					2000	60	0
Myrna Fay 1	Dry	30	10	36.5	50*	45	40
					200	46	48
Myrna Fay 2	Cold storage 3 weeks	6	34	85	50	41	78
Picardy 2	Soaked overnight	11	9	56	100	44	34

* Separate tests were made for each of these samples.

TABLE 2

TETRAZOLIUM RATINGS OF CORMELS OF VARIETY VALERIA STORED FOR VARYING PERIODS OF TIME IN COLD STORAGE AND AT ROOM TEMPERATURE. SEE ALSO FIGURE 5 AND TABLE 3

Date of test	Days in storage	Cormels stored at room temperature: Tetrazolium index 0	1	2	3	4	5	Rating for 20 cormels	Cormels stored at 40° F: Tetrazolium index 0	1	2	3	4	5	Rating for 20 cormels
3/14/51	0	15	15	4	4	1	1	22.0	..	..	..	..	..	..	
3/26/51	12	0	39	0	1	0	0	21.0	0	39	0	1	0	0	21.0
4/4/51	21	0	31	6	3	0	0	22.0	0	31	6	3	0	0	26.0
4/11/51	28	19	16	3	2	0	0	14.0	0	22	3	7	7	1	37.5
4/18/51	35	26	12	2	0	0	0	8.0	0	19	10	8	3	0	37.5
5/18/51	65	29	9	2	0	0	0	6.5	0	5	17	9	5	4	53.0
7/12/51	120	27	13	0	0	0	0	6.5	0	0	4	20	10	6	69.0
8/22/51	161	36	2	1	1	0	0	3.5	3	7	4	14	9	3	54.5

ing of the cormels at any given time with the germination of sister cormels planted at the same time.

Cleaned cormels of four varieties grown at Oceanside, California, and dug in early December, 1950, were obtained from a grower in early March, 1951. These were run through a $^1/_4$-inch screen to eliminate the very small ones and provide a more uniform sample. They were then washed and soaked in water; any rotted ones floating to the top were skimmed off. The four varieties used

were: Elizabeth the Queen, Leading Lady, Margaret Beaton, and Valeria. All had fairly thick husks that were not cracked, with the exception of Margaret Beaton which had approximately 15 per cent cracked husks.

After the cormels were well dried, a tetrazolium test was conducted on each of the four varieties. The cormels were then separated into two equal lots. On March 14, one lot was placed in a cold storage cabinet held at 40° F

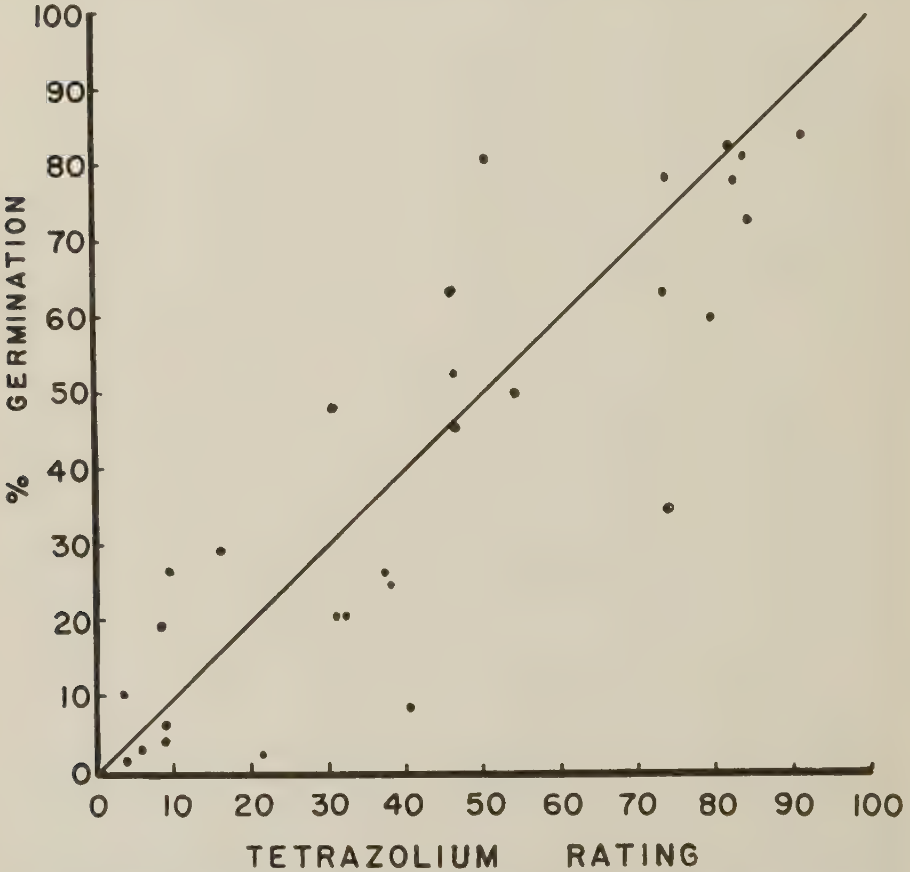

Fig. 7. Scatter diagram showing the correlation of 29 separate tests on a number of different varieties, plotting the per cent germination 60 days after planting against the tetrazolium rating.

and the other left at room temperature (approximately 68° F). Tetrazolium tests of cormels in cold storage and those at room temperature were taken at varying intervals from March through August, 1951. Plantings were made at varying intervals coinciding with the tetrazolium tests. Two hundred cormels of each variety were planted each time in four replicates of 50 each, and germination counts taken weekly. The per cent germination after 60 days was used for comparison with the tetrazolium rating.

Table 2 shows the tetrazolium ratings and germination results for the variety Valeria. Figures 3 through 6 show the results for each of the four

varieties in graphic form. The curves represent the tetrazolium rating as plotted against time in storage at 68° and 40° F. The crosses and circles represent individual germination counts taken 60 days after planting, and are plotted according to the day of planting.

It is apparent from figures 3 to 8 that there is a correlation between the degree of reddening and the germinability of cormels kept in cold storage or

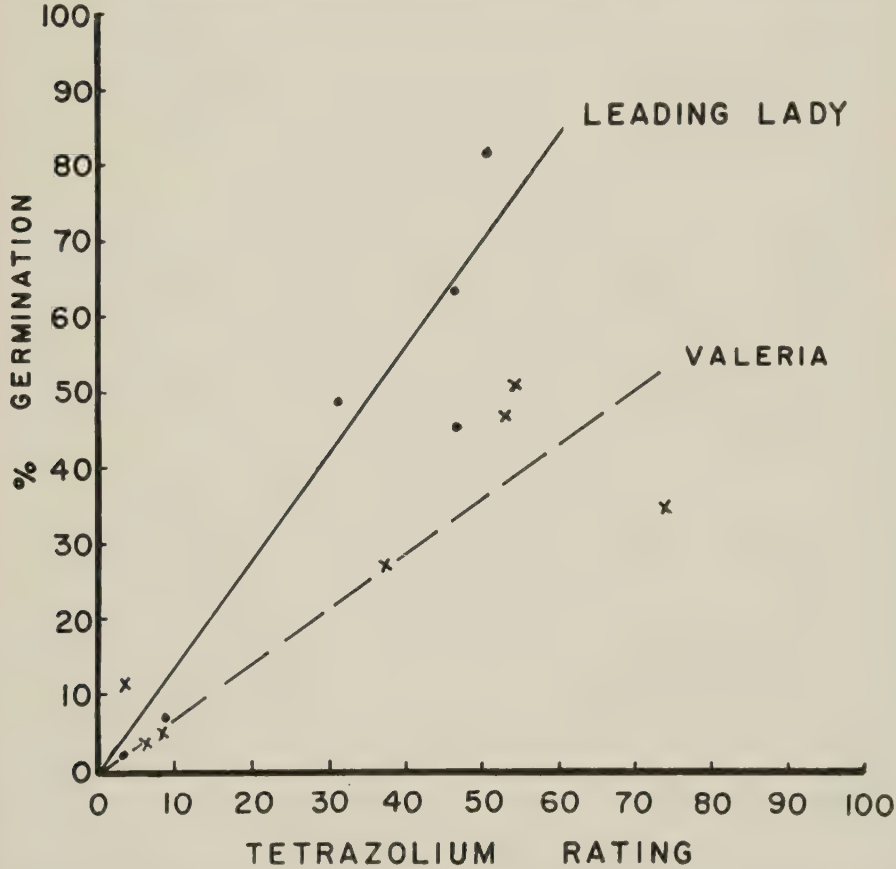

Fig. 8. Correlation of tetrazolium rating and germination of cormels plotted for 12 tests of two varieties, showing their differing slope. These differences are significant at the 5 per cent level. The solid line and the dots represent the variety Leading Lady, the broken line and crosses represent Valeria.

at room temperature. These results also suggest that under conditions similar to those imposed in this trial, germinability can be estimated approximately from the tetrazolium color rating.

Figure 7 shows a scatter diagram of tetrazolium ratings plotted against per cent germination. These represent results of many experiments with a number of varieties. There is an obvious correlation between the two quantities. However, when values for individual varieties are plotted as in figure 8, a closer association is evident within varieties, and there appear to be differences between varieties in the regression of germination on tetrazolium

rating. Statistical analysis of all the data in figure 7 shows differences in the slope of varietal curves to be significant at the 5 per cent level. The values for Leading Lady plotted in figure 8 indicate that, although cormels did not in any trial react with intense reddening, those with moderate tetrazolium ratings gave high percentage germination. The tendency for Valeria was lower germination for equivalent tetrazolium ratings. Subsequent experience has suggested some degree of varietal consistency in these relations. So far, however, varietal differences have not seriously affected the use of the tetrazolium method either in experimental work or in estimating the germinability of commercial lots of cormels. As experience accumulates, varietal characteristics can be taken increasingly into account in estimating germinability from tetrazolium ratings.

TABLE 3

GERMINATION RESULTS OF CORMELS OF VARIETY VALERIA STORED FOR VARYING PERIODS OF TIME IN COLD STORAGE AND AT ROOM TEMPERATURE. SEE ALSO FIGURE 5 AND TABLE 2

Date planted	Days in storage	Cormels stored at room temperature: Per cent germination at indicated days after planting			Cormels stored at 40° F: Per cent germination at indicated days after planting		
		20	40	60	20	40	60
5/18/51	41	0	2	5.0	0	11	27.0
5/24/51	65	..	..	....	4	40	47.0
7/13/51	121	0	1	3.0	9	28	35.0
8/23/51	162	0	2	11.5	7	23	50.5

CONSIDERATIONS IN USING TTC AS A TEST FOR DORMANCY

Although the tetrazolium test can be a very valuable tool for rapidly determining germinability of dormant cormels, certain conditions which may modify the association between color rating and germinability should be understood.

The Effect of Cracking and Thickness of the Outer Husk

During preliminary tetrazolium tests the variety Miss Wisconsin showed a high tetrazolium rating, but when planted would not germinate. If, however, the outer husks were removed the cormels sprouted promptly (table 2 and fig. 9 of Roistacher, Baker, and Bald, 1957). Cormels of this variety had an especially thick, dark outer coat and apparently were unable to germinate because of this covering.

Cormels of the variety Margaret Beaton were segregated into two groups—those whose outer coats appeared naturally cracked and those whose coats were uncracked. A tetrazolium test was taken of each sample, and 150 cormels were planted at the same time. Cormels whose husks had been naturally cracked had a TTC rating of 55.5 (with 75 per cent of the cormels rating 2 and 3), and after 60 days 48 per cent germinated. In the uncracked

cormels the TTC rating was 28 (with 75 per cent of the cormels rating 1) and only 8 per cent germinated after 60 days. The cracked cormels showed a higher TTC rating and a higher per cent germination than uncracked ones. In other experiments, cracking the outer husks invariably resulted in an increased tetrazolium rating. An example may be cited from one experiment where the varieties Elizabeth the Queen and Valeria had a TTC rating of 24 and 22, and 2 weeks after cracking showed a rating of 41.5 and 42.5, respectively. Although corms with cracked husks usually show a higher tetrazolium rating than those with intact husks, recently dug cormels with thin and cracked husks have sometimes had very low ratings.

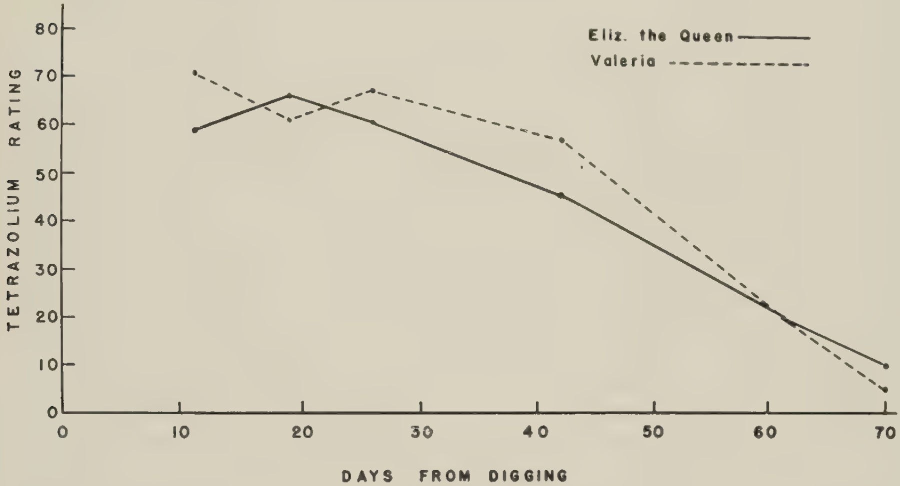

Fig. 9. The decline in tetrazolium reddening of two varieties held at room temperature (68° F) over a period of 70 days following digging. The TTC test does not provide a valid measure of germinability during the early post-digging phase.

The Effect of Time Following Digging

At the time cormels are harvested the tetrazolium test reveals considerable physiological activity in the tissues, which is not associated with germinability. The color pattern differs from that observed later, centering mainly on the parenchymatous tissue and the outer cambial layer of the growing husk. As the husk dries and hardens the color reaction decreases. The decrease in reddening in two varieties of freshly dug cormels over a period of 70 days from digging is shown graphically in figure 9. Further trials over longer periods (Bald and Markley, unpublished data) support the indications of this experiment. As a measure of germinability the TTC color test should be applied only to cormels in the later phases of maturation.

The Effect of Cormel Size

The size of the cormel is definitely related to its level of dormancy. Denny and Miller (1934) showed this to be true when they divided freshly dug cormels of five varieties into four different sizes and found that the large

sizes were less dormant than the small and very small ones and were, therefore, easier to bring out of dormancy by using ethylene chlorohydrin. In tetrazolium tests the larger cormels have repeatedly been found to show a higher degree of reddening than the smaller ones. This was noticed during preliminary tests when a large number of cormels were first peeled and lots of 20 cormels were then cut and placed in separate Petri dishes with TTC. There was an unconscious tendency to select the larger cormels first since they were easier to handle. As a result, the first 20 cormels cut and placed in the first Petri dish usually indicated a higher total rating than those in the second dish. This was remedied by grouping 20 cormels at a time before peeling and cutting. The importance of obtaining a representative sample of uniform-size cormels is evident.

The Rate of Change in Degree of Tetrazolium Reddening

The rate at which cormels lose their dormancy or show an increased tetrazolium rating will vary with the variety and conditions of storage or treatment. Some varieties (*i.e.,* Leading Lady, fig. 4) may show a slow, steady climb in reddening while others (Elizabeth the Queen, fig. 6) may show a sudden increase over a short period of time. It is possible to visualize a situation where a tetrazolium test is taken on cormels while they are undergoing rapid internal change. A germination count taken on sister lots of these cormels 60 days later might show a much higher per cent germination than that predicted from the tetrazolium rating.

DISCUSSION

The original objective in devising the TTC color test for dormancy of gladiolus cormels was to find a quick method for determining the stage in which the cormels were most tolerant to hot-water treatment. Early treated lots which were tested by germination (Roistacher, Baker, and Bald, 1957) had shown that injury from hot-water treatment of dormant cormels was less extensive than to nondormant ones. The most desirable stage for treatment, which seemed to be the condition of maximum dormancy, was estimated by the tetrazolium color reaction applied to small samples of cormels, rather than by prolonged and indecisive germination trials.

The interaction between 2,3,5-triphenyltetrazolium chloride and living tissues produces a color reaction that appears to intensify in proportion to the degree of enzymatic activity. Primarily involved are dehydrogenases, which reduce the colorless tetrazolium salt to an insoluble red formazan (Mattson, Jensen, and Dutcher, 1947; Kun and Abood, 1949; Roberts, 1951; Brodie and Gots, 1952; Ried, 1952; Shelton and Schneider, 1952; Somerson and Morton, 1953; Jámbor, 1954; Jámbor and Mester, 1955; Schatz, Schatz, and Trelawny, 1956; Sorokin and Sorokin, 1956). By periodically rating this red coloration according to a standardized numerical scale, a picture of changing enzymatic activity within the cormel can be obtained. By subjecting cormels to various treatments and studying their tetrazolium reaction it is possible to predict, within limits, the effects of these treatments on germination. Since the TTC reaction in cormels is similar to reactions ob-

served in seeds, it is possible that similar color patterns can be evolved for different seeds and their enzymatic activity studied during dormancy. A low degree of reddening need not merely infer a "loss of the capacity of the seed to germinate" as reported by Lambou (1953) for cotton seed, but could also indicate transitional stages between dormancy and germinability.

Examination of the literature on dormancy, and study of the reaction of gladiolus cormels to this colorimetric test have led to some speculations about the mechanism of their dormancy and germination. Davis (1930) indicated in her work on Xanthium seed that the development of dormancy is closely associated with restriction of oxygen supply by the fruit and seed membranes surrounding them. Barton (1934) found dormancy of Tilia seeds to be due to both an impermeable seed coat and a partially dormant embryo. The seed coats had to be rendered permeable and the embryo after-ripened before germination ensued. Thornton (1945) in a review on seed dormancy concluded that both initial (primary) and induced (secondary) dormancy may be oxygen phenomena. Each "has its inception . . . in the accumulation of intermediate products, formed by partial anaerobic respiration, that act as inhibitors because the oxidation system has been temporarily impaired through an insufficient supply of oxygen." He suggested that high temperature storage augments dormancy because the hydrolyzing system remains active while the oxidation system is inhibited due to insufficient oxygen. Low temperature acts in the reverse fashion.

Many workers (Thimann and Skoog, 1933; Thimann, 1937; Lindner, 1939; Marth, 1942; Hitchcock, 1943; Smith, 1945; Link and Eggers, 1946; Leopold, 1949; Strydom, 1950) have shown that excess auxin can inhibit or prevent vegetative growth. The reduction of free-auxin content should encourage growth or the breaking of dormancy. Thus, Brandes and van Overbeek (1948) showed that breaking of dormancy and multiple sprouting in sugar cane induced by hot-water treatment were related to lowering of the free-auxin level. They suggested that dormancy is regulated by the free-auxin level and that a reduction of it preceded sprouting.

Larsen (1936) obtained an auxin-inactivating substance from the juice of bean seedlings. Larsen (1940) later demonstrated that the inactivation of the auxin by an enzyme occurred only in the presence of oxygen. This was also shown by Tang and Bonner (1947), who found that approximately one molecule of oxygen was consumed for each molecule of indoleacetic acid inactivated. Auxin (indoleacetic acid) inactivation was completely suppressed in the absence of oxygen. Recently Sequeira and Steeves (1954) reported that the leaf drop of coffee caused by *Omphalia flavida* Maubl. and Rang. resulted from auxin inactivation by an oxidative enzyme.

Stewart and Stuart (1942) stored *Lilium longiflorum* bulbs at 77° F under moist conditions for one month. The auxin level decreased until about the time that growth of stem tips and roots began, after which it increased at those points. Strydom (1950) showed that ethylene chlorohydrin broke the dormancy by lowering the free auxin level within gladiolus corms. He suggested a theory of dormancy in the gladiolus corm which is dependent on the amount of free auxin present. He showed that: when the free auxin level is high, corms are dormant; when the free auxin level is at the low or opti-

mum range corms will sprout; and that when the free auxin level has been reduced drastically corms will not sprout, but may be induced to do so if indoleacetic acid is forced into them. When free auxin is reduced to the optimum level and dormancy is broken, growth may be initiated and auxin once again produced in the growing tips.

The normal cycle of dormancy in gladiolus cormels seems to be somewhat as follows: At digging time the tissues of the cormels are still active and the auxin level is presumably high, but not too high for the completion of growth and maturation. When the sheathing husks harden and thicken, oxygen is excluded from the internal cormel tissues, enzymatic activity diminishes, more free auxin accumulates, and growth gradually ceases (fig. 9). A period of dormancy ensues which may last for six months to two years, depending on the thickness of the husks or the rate of their deterioration. Dormancy during this time may be due to the relatively high auxin content within the cormel. One of several factors responsible for continued dormancy appears to be the lack of sufficient oxygen available to the enzyme systems responsible for diminishing free auxin. Dormancy may be effectively broken through partial destruction of free auxin by hot water or ethylene chlorohydrin treatment, and by oxidation after the husks are cracked (fig. 9 in Roistacher, Baker, and Bald, 1957) or the cormels are held in cold storage.

If no dormancy-breaking treatment is applied the cormels will remain dormant until deterioration of the outer husk allows penetration of oxygen, which will reduce the auxin level toward the optimum, break dormancy, and permit embryonic development to proceed.

It would appear that use of the TTC color rating as an indication of cormel germinability rests on the assumptions: 1) that dehydrogenase activity is measured by the intensity of coloration induced under standard conditions in the tissues of sampled cormels, and 2) that dehydrogenase activity and germinability are closely associated. How real this association is will perhaps be revealed by further studies on the enzymes involved in the liberation and degradation of free auxin (Larsen, 1940; Tang and Bonner, 1947). In the meantime, the empirical use of the test has given indispensable aid in the study of the reactions of cormels to hot-water treatment (Roistacher, Baker, and Bald, 1957). It has also been extensively used in later studies on the maturation and development of cormels (Bald and Markley, unpublished data). Probably it could be used also in similar investigations with true seeds.

As a routine technical test of the germinability of cormels, its future is not yet clear. It has been successful when applied by operators aware of its empirical nature and the conditions liable to induce aberrant readings. If, in the future, the enzymatic activity in cormels, as measured by the TTC color test, is linked closely to the initiation and development of root and stem apices, the test may become widely applicable. On the other hand, its practical use may be severely restricted if the color test is found to measure activity which is coincidental, but not directly connected, with these structural changes.

SUMMARY

1. A technique has been presented for classifying various stages of dormancy in the gladiolus cormel. Briefly, the technique consists of longitudinally splitting 20 cormels, and placing the cut surfaces in contact with filter paper saturated with a 1 per cent solution of 2,3,5-triphenyltetrazolium chloride for 4 hours at 70° F in the dark. The cormel will react with the chemical and turn various shades of pink or red depending on its stage of dormancy.

2. A positive correlation has been found between the degree of tetrazolium coloration or reddening and the germination potential of gladiolus cormels.

3. Gladiolus cormels held in cold storage (40° F) showed an increased tetrazolium reddening over a period of 5½ months, and this increase was positively correlated with increased germination of sister cormels planted at various intervals. However, cormels stored at room temperature showed no increase in staining during this period and no increase in germination when planted.

4. Cormels with the outer husks cracked had a higher degree of tetrazolium reddening and a correspondingly higher germination than uncracked ones from the same lot.

5. Freshly harvested cormels showed a reddening reaction to the tetrazolium test which differed in appearance from those of mature cormels. This reddening disappeared completely after a period of time and was apparently associated with the drying and hardening of the outer sheathing husk. At the point of lowest tetrazolium reaction the cormels can be considered most dormant.

6. Larger cormels of a given lot showed a higher degree of tetrazolium reddening than the smaller ones and were, therefore, assumed to be less dormant.

7. The rate at which cormels increased in the degree of tetrazolium reddening varied with different varieties.

8. The tetrazolium test is a useful tool for determining the level of dormancy of gladiolus cormels and the correlated level of tolerance to heat treatment for the eradication of pathogens. Cormels appear to be most tolerant to the heat treatment when at maximum dormancy.

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